EXHIBIT C

Project No. 414
Book No. 1003 TITLE Primers for Human Endostatin PCR

From Page No. [redacted]

Human α1 (XVIII) Collagen Gene Sequence Genbank Accession # L22548
This actual sequence alignment with mouse came from paper
Cloned cDNA & Genomic DNA Encoding Human Type XVIII Collagen
and Localization of the α1(XVIII) Collagen Gene to Mitotic Chromosome
16 and Human Chromosome 21 Suk K. DH. et al Genomics 19 pg 494-499 (1994)

Book 1003 pg. 110 [redacted] ET AL

Primers made are highlighted in hot pink for endostatin

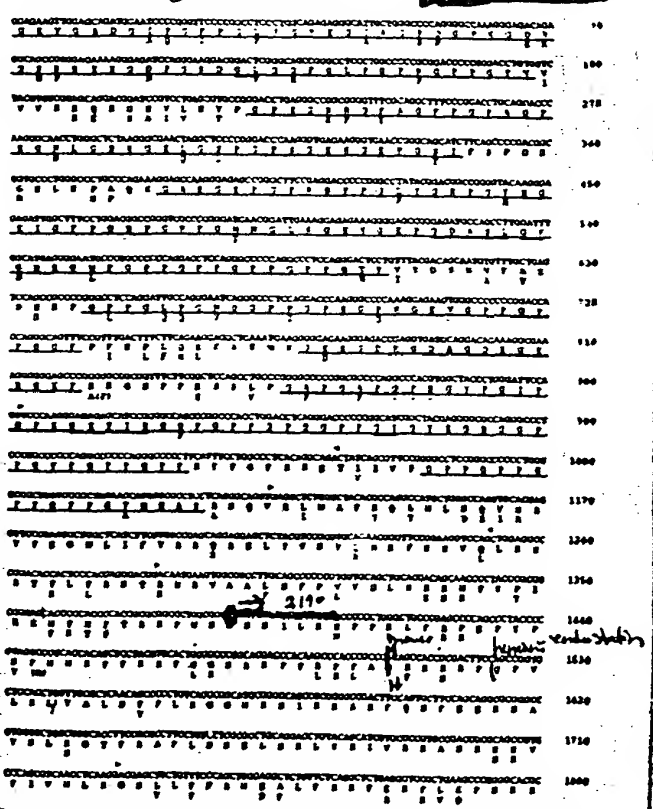


FIG. 2. The combined nucleotide sequence of human cDNAs and the conceptual amino acid sequence of human α1(XVIII) collagen are compared with the amino acid sequence of mouse α1(XVIII) collagen. To obtain maximum identity, six gaps (—) of nonidentical amino acids were introduced in the mouse sequence. Only residues that are different between the human and mouse polypeptides are shown in the mouse line. The sequence defining triple-helical domains are underlined. Five asparaginyl-glycyl sequences are double underlined. As explained in the text, we have identified the boundaries of four exons by partial sequencing of genomic fragments. The 5′ boundaries of these exons are indicated by arrows pointing to the right, placed above the most 5′ nucleotide of the exon. The 3′ boundaries of the exons are indicated by arrowheads pointing to the left, placed above the most 3′ nucleotide of the exon. Sequences between the four defined exons may represent single exons or additional exons. Splicing of the genomic clone will, however, be necessary to reconstitute them. Four conserved residues are underscored by bold letters and the two poly-tryptophan tracts are underlined by a series of A. The transmembrane stop codon is underscored by an asterisk.

To Page No. [redacted]

Witnessed & Understood by me, [redacted]

Date [redacted]

Invented by [redacted]

Recorded by [redacted]

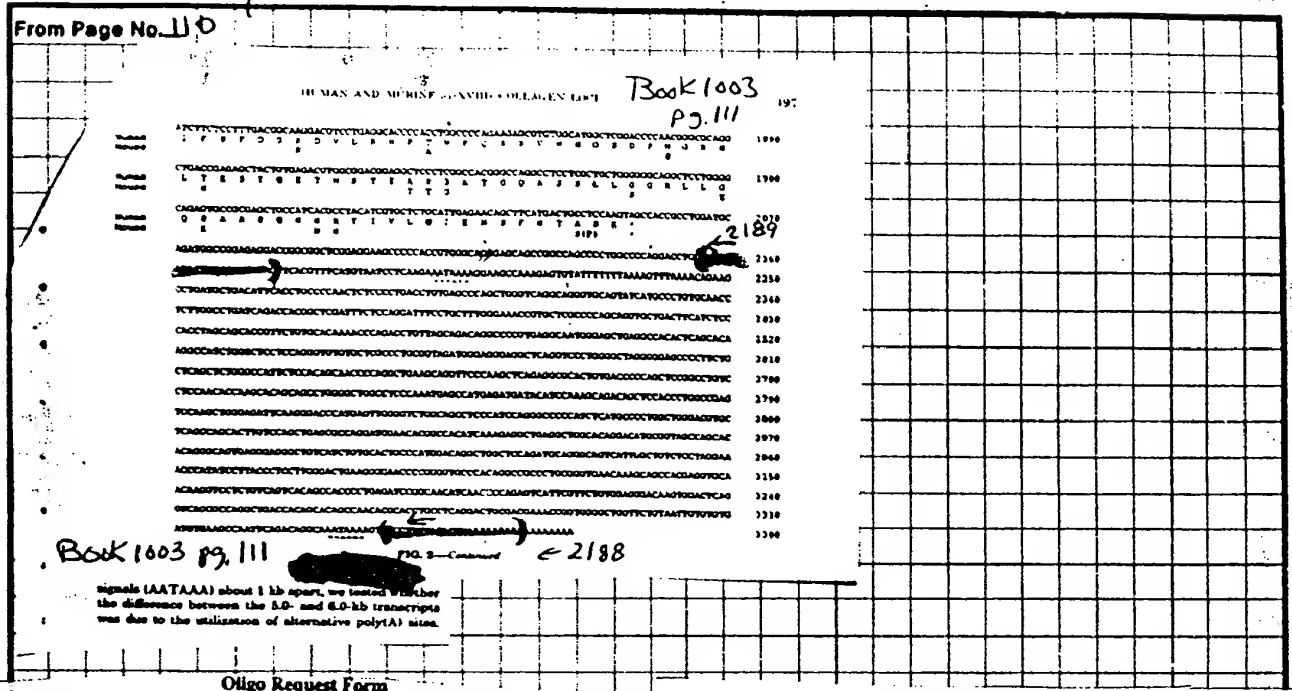
Date [redacted]

TITLE Primers for Human Endostatin PCR

Project No. 414

Book No. 1003

From Page No. 110



signals (AATAAA) about 1 kb apart, we tested whether the difference between the 5.0- and 6.0-kb transcripts was due to the utilization of alternative polyA) sites.

Oligo Request Form

Name: Sandrina Phipps | Project Code: 414 | Date: [redacted]

Length of Oligo: 22 Antisense

Purification: ☐ Unpurified ☐ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*

Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)

Sequence written 5' to 3' in triplets:

CTA TAC AGG AAA GTA TGG CAG C ___ ___

Total #G 6 #A 8 #T 4 #C 4

2189 0.7 mg/ml

Purpose: PCR GENE SYNTHESIS

SSP [redacted] Book 1003 pg. 111

Oligo Request Form

Name: Sandrina Phipps | Project Code: 414 | Date: [redacted]

Length of Oligo: Antisense

Purification: ☐ Unpurified ☐ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*

Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)

Sequence written 5' to 3' in triplets:

TTT TTT TTT CAG TGT AAA AGG TC ___ ___

Total #G 4 #A 5 #T 12 #C 2 2188 (2.6 mg/ml)

Purpose: PCR GENE SYNTHESIS

SSP [redacted] Book 1003 pg. 111

To Page No. 112

Witnessed & Understood by me, Lisa M. Clarke

Date [redacted]

Invented by Sandrina Phipps

Recorded by

Date [redacted]

Project No. 414
Book No. 1003
TITLE Primers for Human Endostatin

From Page No. 111 [redacted]

Oligo Request Form

Name: Sandrina Phipps	Project Code: 414	Date: [redacted]

Length of Oligo: 19 — sense

Purification: ☐ Unpurified ☐ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*

Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)

Sequence written 5' to 3' in triplets:

CAG ATG ACA TCC TGG CCA G

Total #G 5 #A 5 #T 3 #C 6 2190 2.3mg/ml

Purpose: PCR gene synthesis Book 1003 pg. 112 SSP. [redacted]

Oligo Request Form

Name: Sandrina Phipps	Project Code: 414	Date: [redacted]

Length of Oligo: 17 — A.S.

Purification: ☐ Unpurified ☐ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*

Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)

Sequence written 5' to 3' in triplets:

III III III III III II

Total #G ___ #A ___ #T 12 #C ___ 2187 2.4mg/ml

Purpose: PCR Book 1003 pg. 112 SSP. [redacted]

[redacted]

Sandrina Phipps

[redacted]

TCID 50 spreadsheet for AdnBglX SSP1-98 from page 38. Calculated titer 5.18X10^8 PFU/ml. This is very low titer compared to particles of 6.5X10^11 particles/ml. It also was identified by PCR that this virus does not appear to have the site; primer does not amplify to product. I probably will not use this virus.

Dilutions	Number of Infected Wells	Number of Uninfected Wells	Total Number Infected	Total Numbe Uninfected	Percent Total Infect	Above 50%	Percent Above 50%	Percent Below 50%
1.00E-06	12	0	21	0	100.00%	TRUE	0.00%	0.00
1.00E-07	7	5	9	5	64.29%	TRUE	64.29%	0.00
1.00E-08	1	11	2	16	11.11%	FALSE	0.00%	11.11
1.00E-09	1	11	1	27	3.57%	FALSE	0.00%	0.00
1.00E-10	0	12	0	39	0.00%	FALSE	0.00%	0.00
1.00E-11	0	12	0	51	0.00%	FALSE	0.00%	0.00
1.00E-12	0	12	0	63	0.00%	FALSE	0.00%	0.00

Book 1003 page 112 SSP [redacted]

# of Wells	12						0.64285714	0.1111111
mls/well	0.02							
Prop. Dist.	0.268856718							
Log TCID50	-7.268856718							
TCID50	5.39E-08							
1/TCID50	1.86E+07			PFU/Ml	AVG PFU/M	Titer PFU/ML		
			#2	1.94E+08	3.44E+08			
TCID50/ml	9.28E+08			4.93E+08		5.18E+08		
			#1	743279968	6.92E+08			
pfu/ml	6.40E+08			640436111				

To Page N

Witnessed & Understood by me, Lucia M. Clark — Date [redacted]

Invented by

Recorded by Sandrina Phipps — Date [redacted]

TITLE

From P

TITLE RTPCR for Human Endostatin

Project No. 414
Book No. 1003

From Page No. [redacted]

Plated HeLa S cells 3 plates to set up transfection efficiency
for pAmbg14 plasmid. pan 32

47) 35.66x10⁴ cells/ml Need 7.2x10⁶ cells / 35.66x10⁴ cells/ml
30 } Need 4x10⁵ cells / 4ml well. = 21ml
30) I used all of 20ml. These will be slightly less than 4x10⁵ cells on each well.

20ml + 55 ml media = plate 4ml/well.

Received oligos for Human + Canine Endostatin dated Jan 8, 1998

For human endostatin PCR of Human Liver Poly A RNA we need to use primers 2190 - sense, 2188 & 2189 antisense

2190 → 2.3mg/ml - mwt = 5793.8g/mole

$\frac{2.3 g/liter}{5793.8 g/mole}$ = .00040 x $\frac{1000 mMoles}{moles}$ = 0.4000 x $\frac{1000 uMoles}{mMoles}$ = 400uM

Made 1uM dilution 1.25 ul / 500ul

2188 $\frac{2.6 g/liter}{7046.6 g/mole}$ = .00037 x $\frac{1000 mMoles}{moles}$ = 0.37 x $\frac{1000 uMoles}{mMoles}$ = 370uM

Made 1uM dilution 1.35ul / 500ul

2189 $\frac{1.7 g/liter}{6787.4 g/mole}$ = .0001 $\frac{moles}{liter}$ x $\frac{1000 mMoles}{moles}$ x $\frac{1000 uMoles}{mMoles}$ = 103uM

1uM = 5ul / 500ul H_2O

4ul $MgCl_2$ (25mM)
2ul 10x PCR Buffer II
2ul H_2O
8ul dNTPs (10mM)
1ul RNASE Inhibitor
1ul MuLV Reverse Transcriptase
1ul oligo d(T)16
1ul human Liver RNA (100ng/ul)
1ul PFU (2.5u/ul)

Human Liver Poly A⁺ RNA - purified guanidinium thiocyanate → purified 2x oligo (dT) cellulose columns
Lot # 6120305 Clontech (1ug/ul)
5ug total received.

10min @ room temp
15min @ 42°C
5min @ 99°C
5min @ 5°C
4°C Hold Cycle

To Page No. 126

Witnessed & Understood by me, [signature]
Date [redacted]
Invented by
Recorded by [signature]
Date [redacted]

Project No. 414
Book No. 1003 TITLE PCR of Human Endostatin

From Page No. 125 [redacted]

RTPCR After 4°C hold. EtOH precipitated 2ul 3M NaAC → 400ul EtOH. Store @ -70°C for 15min. Centrifuged 30min @ 4°C Washed in 70% EtOH. Resuspended pellet in 74.5ul H_2O

Setup PCR rxn with PFU

1X Buffer	10ul	(10X PFU Buffer)	95°C 3min
1uM primer	1ul	(1uM) 2190	Hold @ 80°C for 3min
1uM primer	1ul	(1uM) 2188 & 2189	35 cycles 1min @ 95°C
1.5mM $MgCl_2$	6ul	(25mM)	2min @ 60°C
0.7mM dNTPs	7.5ul	(10mM)	2min 20sec @ 72°C
dH_2O	74.5 ~~68.5~~ ul		Extend 10min
PFU enzyme	1ul	(2.5u/ul)	Hold @ 4°C
	100ul		gel picture pg 127

PCR (21 88/90) → 2.0Kb (2189/90 → 1.790Kb)

Setup 10ml culture glycerol of SQ11 from Qiang Kang to make Calcium prep.

Sandra Phipps [redacted]

[redacted] Set up Transfection Efficiency of Hep3B cells with Lipofectamine plus reagent.

	DNA		Plus Reagent	Optimem	Lipofectamine	Optimem	Make 3 RXNs
A	3ul (1ug/ul)	pAvnBgx	18ul	300ul	6ul	300ul	total amt on plate 209ul
B	3ul	"	18	"	12ul	300ul	211ul
D	6ul	"	30	"	15ul	"	217ul
E	6ul	"	31ul	"	30ul	"	222ul
F	3ul	"	24ul	"	15ul	"	214ul
G	6ul	"	—	"	15ul	"	207ul

① Incubate DNA plus reagent serum-free media 15min RT
② Mix lipofectamine & serum-free media for pre complex 15min RT
③ Wash cells 1X with optimem media (1ml)
④ Add 0.8ml of optimem media overlay the precomplex/lipofectamine reagent ~200ul.
⑤ Incubate 1 set of 6 well plates 3 1/2 hr. remove transfection & add 4ml EMEM+10%FBS. 1 set of plates left transfection mix on ON, then will change media

To Page No. 128

Witnessed & Understood by me, Yoni M Clark | Date [redacted] | Invented by | Recorded by Sandra Phipps | Date [redacted]

Project No. 414
Book No. 1003 TITLE RT PCR Human Endostatin / Plasmid Prep

From Page No. 127 [redacted] SQ1

Processed Bulk plasmid prep of pSQ1 - as described on page 61.
Processed to Overlay import Cesium gradient Spinning ON @ 55K.

Setup new RT-PCR reaction for human Endostatin since no amplification products were visible the original reaction.
I will use random hexamer & downstream primer 2188 this time for priming (instead of oligo dT)

4ul $MgCl_2$ (25mM)	10min @ room temp
2ul 10X PCR Buffer	15min @ 42°C
2ul Dep. H_2O	5min @ 99°C
8ul dNTPs (10mM)	5min @ 5°C
1ul RNase Inhibitor	Hold @ 4°C
1ul MuLV reverse transcriptase	
1ul Random Hexamer or 2188	
1ul RNA (1ug) - Human liver poly A+	

20ul rxn.

EtOH ppt 2ul NaOAC + 300ul EtOH ppt → Resuspend 20 ul

Setup PCR (cDNA)	3min @ 95°C
20ul cDNA	80°C 3min. - Add PFU (2.5u) 1ul
10ul 10x PFU Buffer	35 Cycles 1min @ 95°C
1ul 2190	2min @ 58°C
1ul 2189	~~2min @ 20°C~~ 2min 20°C 72°C
7.5ul dNTPs	Extend 15min @ 72°C
59.5ul H_2O	Hold @ 4°C ON
1.0ul PFU Hot Start	

Poured 1% Seaplaque gel to run products ON.
Did a zip lock for Clone NO
From pg 126
Added 4ml of fresh EMEM + 10% FBS to transfected Vap 3B cells Which were left ON ≃ 24 Hr post transfection in Lipofectamine.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
Lin M Chby	[redacted]	Recorded by Sandra Phipps	[redacted]

TITLE Protocol for PerkinElmer RT-PCR

Project No. 414

Book N . 1003

From Page N . [redacted]

PROTOCOLS FOR REVERSE TRANSCRIPTION OF RNA AND PCR AMPLIFICATION OF cDNA

[illegible]

Book 1003 page 129 55P[redacted]

PERKIN ELMER

PERKIN ELMER

To Page No.

Witnessed & Understood by me, Lim M Clarke

Date [redacted]

Invented by

Date [redacted]

Recorded by Sandline Phipps

Project No. 414

TITLE RT PCR of Human Endostatin Book No. 003

From Page No. __ [redacted]

I will use (pAW109) RNA control Perkin Elmer Kit. Refer to protocol on page 129

I will use random hexamer & downstream primer for pAW109 RNA Control DM152

and 2188 for human endostatin. I will also use Taq + PFU enzyme.

		PFU	Taq
25mM MgCl2	4ul	✓	
10mM dNTPs	8ul	✓	
10x PCR II Buffer	2ul	✓	
H2O	2ul	✓	
RNAse inhibitor	1ul	✓	
Random Hexamer	1ul	or 1ul 20uM 2188 or 1ul DM152 ✓	
RT	1ul	✓	
	20ul rxn.		

10 min room temp.
15 min @ 42°C
~~5~~ 5 min @ 99°C
5 min @ 5°C
Hold @ 4°C

I accidentally started Hot Start program instead of RT-PCR program. I realized instantly however tubes had briefly warmed to 95°C. I added fresh RT enzyme.

After RT precipitated (EtOH) PFU rxns because need to change buffer.

Set up Taq master mix

78ul - master mix for Endostatin RNA ... 1 pAW109 control RNA

4ul MgCl2 (25mM)
8ul PCR Buffer 2
45.5ul H2O ... 65.5ul H2O
10ul 2190 (1uM) to primer conc ... or 1ul DM152
10ul 2189 (1uM) " " ... 1ul DM151
100ul

Add 0.5ul ampliTaq hot start @ 80°C

3 min @ 95°C
3 min @ 80°C - Add Taq
35 cycles 1 min @ 95°C
1 min @ 60°C
1 min 31 sec @ 72°C
Extend 10 min @ 72°C
Hold @ 4°C

When I programmed machine @ cycle 4 I left on hold @ 60°C. The rxns set @ 60° for 2 1/2 Hrs before starting cycles as described above

Lori Clarke froze samples @ -20°C [redacted]

I went to set up PFU PCR - I added perkin Elmer buffer II instead of PFU Buffer. I EtOH ppt over the weekend.

To Page No. 134

Witnessed & Understood by me, Lori M Clarke Date [redacted] Invented by Recorded by Sandine Phipp Date [redacted]

134

Project No. 414
Book No. 1003
TITLE RT PCR / Preparation for Homologous Recombination of AVE1Y2 + AvnBglx Virus

From Page No. 133 [redacted]

I EtoH ppt the RT PCR reactions from page 133. Setup PFU PCR rxns (SQ)

20ul cDNA (H2O) for Ondo Stdn RNA
10ul PCR Buffer (PFU)
10ul 2190
10ul 2189
7.5ul dNTPs (10mM)
39.5ul H2O
1ul PFU @ Hot Start (2.5u/ul)
100ul rxn. overlay mineral oil

PAW109
1ul DM152
1ul DM151
39.5ul H2O
I accidentally added 10ul of prim 2189+2190 also.

3min @ 95°C
3min @ 80°C - Add PFU
35 cycles 1min @ 95°C
1min @ 60°C
3min @ 72°C
Hold @ 72°C for 10min
Hold @ 4°C. ON

Setup digests of pAVE1X2 to use in transfections on Hep3B cells for Homologous recombination studies. Need to linearize plasmid.

6ug (1.6ug/ul) 3.75ul
5.0ul 10X BSA
5.0ul Buffer 3
3.0ul NotI
50.5ul H2O
Incubate @ 37°C ON.

Plated 8 6well plates of Hep3B cells @ ≈ 4.0x10^5 cells/well for transfection of Homologous recombination Story.

49
57 } 55.7x10^4 cells/ml Need 21x10^7 cells 1x10^5 cells/ml x 210ml =
61 Added media up to 210ml. I probably do not have exactly 4.0x10^5/well, but its very close.
Plated 1 T150 1:20 passage 34

To Page No.

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

Project No. 414

TITLE Continuation of Homologous Recombination of Book No. 1003

From Page No. 142 [redacted] pAVEILX2 + SQ1 Virus

I split passage 32 S8 cells plated 1x10⁴ cells/well use 96 well plate to do TCID50 titer of SQ1-P2 virus from Vicky North which I used in Homologous recombination study.

173 }
109 } 137.6x10⁴ cells/ml
131 }

Need 5x10⁴ cells/ml x 22ml = 1.1x10⁶ cells / 137.6x10⁴ cells/ml = 0.8ml + 21.2ml media + 66ul 100uM dexamethasone

Plated 200ul of cells/well. 1 96 well plate.

[redacted] Sandrina Phipps

[redacted]

I harvested 24hr time pt for homologous recombination study of SQ1-P2/pAVEILX2 as described on page 142. Snap froze pellets on Sat June 20th collect 72hr time pt.

I added 6x10⁹ particles/ml to 96 well S8 plate to look for virus production this is to confirm that the SQP2 virus used for homologous recombination study is active.
Put 10ul of 6x10⁹ particles/ml 1st well diluted down the plate 1:10 Left bottom row blank. go to page 145

Stained the pAVnBglx transfection control for homologous recombination transfection efficiency.
Washed 2X wells PBS. Fixed with glutaldehyde/formaldehyde solution 5min @ room temp. Followed protocol page 58 Book 893.

Set up RT rxn for human endostatin

		PE4	
	25mM MgCl2	4ul	10min @ room temp
	10mM dNTPs	8ul	15min @ 42°C
	10X PCR Buffer	2ul	5min @ 99°C
	H2O	2ul	5min @ 5°C
	RNase Inhibitor	1ul	Hold @ 4°C
RNA: PAW109 1ul	Random Hexamer 2188 OM152	1ul	
100ng human Liver poly A RNA	RT	1ul	

To Page No. 144

Witnessed & Understood by me, Lin M Clark
Date [redacted]
Invented by
Recorded by Sandrina Phipps
Date [redacted]

Project No. 414
Book No. 1003 TITLE RT-PCR Endostatin

From Page No. 143 [redacted]

Ethanol precipitate RT-rxn added 2ul 3M Na Acetate + 40ul cold EtOH
Precipitate @ -70°C for 15min → Centrifuge 30min @ 4°C
Wash 1X 70% EtOH Set up PCR RXNS The DM152 primer PAD109 control tube
20ul cDNA ✓ busted - will have no PCR rxn.
10ul PCR Buffer Clontech PFU ✓ 3min @ 95°C
8ul dNTPS 3min @ 80°C Add PFU
10ul 2190 ✓ For PAD109 control use 1ul DM152 ✓ 35 cycles / 1min @ 95°C
10ul 2189 ✓ 1ul DM151 ✓ 1min @ 60°C
39ul H_2O ✓ 57ul H_2O ✓ 3min @ 72°C
1ul PFU Hot Start (2.5u/ul) Extend 15min @ 72
100ul Rxn. Hold @ 4°C

Gel picture 145

Plated 293 Cells → passage 11 to set up 2 6 well plates for transfection of pAVEla101x

99
68 } 79.7x10^4 cells/ml need 1x10^5 cells/ml = 4ml/well = 4x10^5 cells/well
72 1.0x10^5 cells/ml & 55ml = 5.5x10^6 cells / 79.7x10^5 cells/ml

Need 6.9ml of cells + 48.1ml Richter's + 10% FBS

Digested SQ1 cesium DNA
30ug = 40ul (0.76ug/ul) Incubate @ 65°C ON
5ul Buffer 4
2.5ul BSTB1 - Same as SfuI
2.5ul H_2O
50ul

To Page No.

Witnessed & Understood by me, Zevin M Clark Date [redacted] Invented by Recorded by Sandra Rupp Date [redacted]

Project No. [illegible]
Book No. [illegible] TITLE [illegible]

From page no. [redacted]

Setup [illegible] reaction [illegible]
- 4ul 25mM MgCl2 — 1 hr @ room temp
- 3ul 1mM dNTPs — 15min @ 37°C
- 2ul Rl buffer II — 5 min @ 99°C
- 2ul H2O — Hold @ 4°C
- 1ul RNase inhibitor
- 1ul Random hexamer
  - a 2188 (5um)
- 1ul RT
- 1ul RNA (poly A [illegible])
- 20ul

EtOH precipitated 20ul 3M NaAc + 330ul EtOH

Setup PCR Reaction — 3min @ 95°C
- 39ul H2O + [illegible] — 3min @ 80°C - Add PFU
- 10ul [illegible] Buffer (PFU) — 35 cycles 1min @ 95°C
- 10ul 2190 [illegible] — 1min @ 55°C
- 10ul 2189 [illegible] — 3min @ 72°C
- 30ul dNTPs (2.5mM each) — Extend 15min @ 72°C
- 98ul – [illegible] add 2ul (2.5u/ul PFU) — Hold @ 4°C O/N

[illegible] amplification [illegible] from pg 163. It appears the [illegible] → may have contamination in the [illegible]. Will filter it and amplify supernatant.

Made 2 T75 [illegible] S8 cells + dex 0.3uM passage 37 to amplify pNEB [illegible]
Plated 1x10^6 cells/T75 → 1.15ml of 1.3x10^6 cells/ml. [illegible] had already trypsinized & counted cells.
Plated 6 well plate 1x10^4 cells/ml x 40ml = 4.0x10^5 cells / 1.3x10^6 cells
Need 0.308 ml
Plated 4ml / well 4x10^5 cells / well

Cl broke out new vial of S8 cells passage 17 into T75 flask (frozen on 11/7/98 SSP)

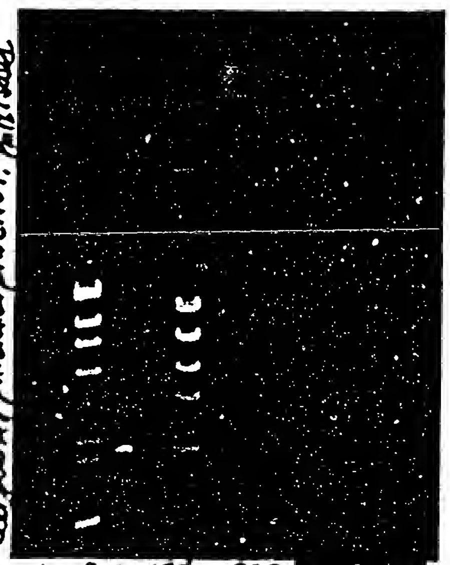
To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	[redacted]	Recorded by [signature]	[redacted]

178 Project No. 414
Book No. 1003 TITLE Cloning of pAVE1a09LXi and Endostatin PCR-Script

From Page No. [redacted]

Set up ligation for pAVE1LX2/pAVE1a09: to make pAVE1a09LXi.
Ran check gel to look @ gel purified fragments.



Book 1003 Pg 178 SSP [redacted]

Lanes 1 mix λHind III/φX HaeIII
2 10ul pAVE1LX2 PmlI/SalI
3 10ul pAVE1a09: PmlI/SalI
4 High mass ladder 4ul
5-10 BLK

7ul pAVE1LX2 PmlI/SalI 6.3kb
11ul pAVE1a09: PmlI/SalI 3.6kb
2ul Ligation Buffer
1ul ligase
21ul Incubate @ 15°C O/N.

Set up PCR-Script Cloning of Endostatin
I made 20ul rxn instead of 10ul reaction as in protocol because my [illegible]
1ul PCR-Script Amp SK(+) (10ng/ul)
2ul 10x Reaction Buffer
~~0.5ul~~ 1.0ul 10mM rATP
2.0ul SrfI
2ul T4 DNA ligase
12ul Endostatin human PCR product 790bp
20ul

Incubate @ room temp 25°C for 1 Hr.
Heat @ 65°C 10min.
Place on ice until do ligation.

Go to page 180

To Page No. 180

Witnessed & Understood by me.	Date	Invented by	Date
[illegible signature]	[redacted]	Recorded by [illegible signature]	[redacted]

180

Project No. 414
Book No. 1003
TITLE Ligation + Transformation Protocol for PCR Script - Strat[illegible]

From Page No. 179 [redacted]

I picked 15 colonies from PCR Script cloning kit transformation done on [redacted] protocol below. 3ml cultures grew @ 37°C O.N.

Polishing the Purified PCR Products

Polish the ends of purified PCR products generated with either *Taq* DNA polymerase or other low-fidelity DNA polymerases as indicated in the following protocol.

Note *Pfu DNA polymerase-generated PCR products do not require polishing. Proceed directly to Inserting the PCR Products into the pPCR-Script Amp SK(+) Cloning Vector.* I had used PFU

1. To prepare the polishing reaction, add the following components *in order* to a 0.5-ml microcentrifuge tube:

   10 μl of the purified PCR product
   1 μl of 10 mM dNTP mix (2.5 mM each)
   1.3 μl of 10x polishing buffer
   1 μl of cloned *Pfu* DNA polymerase (0.5 U)

2. Mix the polishing reaction gently and add a 20-μl mineral oil overlay.
3. Incubate the polishing reaction for 30 minutes at 72°C.
4. Add an aliquot of the polished PCR product directly to the ligation reaction (see *Inserting the PCR Products into the pPCR-Script Amp SK(+) Cloning Vector*) or store the polished PCR products at 4°C until ready for further use.

Inserting the PCR Products into the pPCR-Script Cam SK(+) Cloning Vector

Calculating the Insert-to-Vector Molar Ratio

This kit requires a high insert-to-vector molar ratio for ligation, higher than the molar ratios used in many other cloning procedures. The ideal molar ratio of insert-to-vector DNA is variable. The control ligation in this kit is optimized to use an ideal test insert-to-vector ratio of 100:1. For the sample DNA, a range from 40:1 to 100:1 insert-to-vector ratio is recommended. Use the following equation to optimize conditions for the insert:

$$X \text{ ng of PCR product} = \frac{(\text{number of base pairs of PCR product})(10 \text{ ng of pPCR Script cloning vector})}{2961 \text{ bp of pPCR Script cloning vector}}$$

where *X* is the quantity of PCR product (in nanograms) required for a 1:1 insert-to-vector molar ratio. The following table provides examples of optimal insert-to-vector molar ratios calculated using the above equation:

Size of PCR product (bp)	Quantity of PCR product (ng), 40:1–100:1
250	35–85
500	65–165
750	100–250
1000	130–330
1500	200–500
2000	265–660
3000	400–990

Book 1003 pg 180 SSP [redacted]

Ligating the Insert

1. To prepare the ligation reaction, add the following components *in order* in a 0.5-μl microcentrifuge tube:

   1 μl of the pPCR-Script Amp SK(+) cloning vector (10 ng/μl)
   1 μl of PCR-Script 10x reaction buffer
   0.5 μl of 10 mM rATP
   2–4 μl of the blunt-ended PCR product or 4 μl of the control PCR insert
   1 μl of *Srf* I restriction enzyme (5 U/μl)
   1 μl of T4 DNA ligase
   Distilled water (dH_2O) to a final volume of 10 μl

2. Mix the ligation reaction gently and incubate this reaction for 1 hour at room temperature.
3. Heat the ligation reaction for 10 minutes at 65°C.
4. Store the ligation reaction on ice until ready to use to transform the Epicurian Coli® XL1-Blue MRF' Kan supercompetent cells.

TRANSFORMATION GUIDELINES

Supercompetent Cells

Epicurian Coli XL1-Blue MRF' Kan supercompetent cells are very sensitive to small variations in temperature and must be stored at the bottom of a –80°C freezer. Transferring tubes from one freezer to another may result in a loss of efficiency. Store the Epicurian Coli XL1-Blue MRF' Kan supercompetent cells on ice at all times while aliquoting. It is essential that the Falcon 2059 polypropylene tubes are placed on ice before the supercompetent cells are thawed and that the supercompetent cells are aliquoted directly into the prechilled polypropylene tubes. Pipet the remaining supercompetent cells into 40-μl aliquots and freeze the aliquots at –80°C. Do not pass the frozen supercompetent cells through more than one freeze-thaw cycle.

Note *The efficiency of the Epicurian Coli XL1-Blue MRF' Kan supercompetent cells should be ≥5 × 10^8 cfu/μg. (Approximately 500 colonies are expected when 1 μl of supercompetent cells are plated.)*

Use of Falcon 2059 Polypropylene Tubes

It is important to use Falcon 2059 polypropylene tubes for the *Transformation Protocol*, because other tubes may be degraded by β-mercaptoethanol. Additionally, the critical incubation period during heat pulsing is calculated for the thickness and shape (i.e., round bottom) of the Falcon 2059 polypropylene tube.

Use of β-Mercaptoethanol

β-Mercaptoethanol increases transformation efficiencies two- to threefold. This kit includes prediluted, ready-to-use β-mercaptoethanol.

Length of the Heat-Pulse

Optimal transformation efficiencies are observed when transformation reactions are heat-pulsed for 45–50 seconds. Stratagene recommends heat-pulsing for at least 45 seconds to allow for variability in the duration of heat-pulse. Transformation efficiencies decrease sharply when heat-pulsed for <45 seconds or for >60 seconds.

Book 1003 pg 180 SSP. [redacted]

SSP 181

Witnessed & Understood by me, Lori M Clark
Date [redacted]
Invented by
Recorded by Sardine Phipps
Date [redacted]

TITLE Transformation Protocol (pPCR-Script - Stratagene) Project No. 414 Book No. 1003 181

From Page No. 180 [redacted] SSP

TRANSFORMATION PROTOCOL

1. Thaw the Epicurian Coli XL1-Blue MRF' Kan supercompetent cells on ice.

2. Gently mix the Epicurian Coli XL1-Blue MRF' Kan supercompetent cells. Aliquot 40 µl of the supercompetent cells into a prechilled 15-ml Falcon 2059 polypropylene tube for the experimental transformation reaction. For transformation of the ligation reaction containing the control PCR insert, aliquot 40 µl of the supercompetent cells into a prechilled Falcon 2059 tube. For a control transformation using the pUC18 control plasmid, aliquot 20 µl of the supercompetent cells into a prechilled Falcon 2059 tube.

3. To prepare the experimental transformation reactions and the transformation containing the control PCR insert, add 0.7 µl of β-mercaptoethanol to the polypropylene tubes containing the supercompetent cells to yield a final concentration of 25 mM. For the transformation reaction with the pUC18 control plasmid, dilute 1 µl of the provided 1.44 M β-mercaptoethanol with 1 µl of dH$_2$O, and add 0.7 µl of the diluted β-mercaptoethanol to the appropriate tube of supercompetent cells.

4. Swirl the transformation reactions gently. Incubate the transformation reactions on ice for 10 minutes, swirling the reactions gently every 2 minutes.

5. Add 2 µl of the experimental ligation reactions from step 4 of *Ligating the Insert* to the experimental transformation reactions and swirl the reactions gently. Add 2 µl of the ligation reaction containing the control PCR insert from step 4 of *Ligating the Insert* to the appropriate transformation reaction and swirl gently. Add 1 µl (0.1 ng) of the pUC18 control plasmid to the appropriate transformation reaction and swirl gently.

6. Incubate the transformation reactions on ice for 30 minutes.

   Note *Prepare the SOC medium and equilibrate the medium to 42°C.*

7. Heat-pulse the transformation reactions in a 42°C water bath for 45 seconds. The duration of the heat-pulse is critical for optimal transformation efficiencies (see *Length of the Heat-Pulse*).

8. Incubate the transformation reactions on ice for 2 minutes.

9. Add 0.45 ml of the prepared SOC medium to each transformation reaction and incubate the reactions at 37°C for 1 hour with shaking at 225–250 rpm.

10. Prepare the plates for color selection by spreading 20 µl of 0.2 M IPTG and 20 µl of 10% (v/v) X-gal on all agar plates 30 minutes prior to plating the transformation reactions.

    Note *Avoid mixing the IPTG and the X-gal before spreading them on the agar plates because these chemicals precipitate when combined. Prepare X-gal in dimethylformamide (DMF). Prepare IPTG in sterile dH$_2$O.*

11. Plate the experimental transformation reaction, the transformation containing the control PCR insert, and the pUC18 control transformation as follows:

    a. Use a sterile spreader to plate 200 µl (or less) of the experimental transformation reaction onto LB–ampicillin agar plates.[1]

       Note *The cells may be concentrated by centrifuging at 1000 rpm for 10 minutes if desired. Resuspend the pellet in 200 µl of NZY⁺ broth and plate.*

    b. Use a sterile spreader to plate 50 µl of the transformation reaction containing the control PCR insert onto a LB–ampicillin agar plate and 50 µl of the transformation reaction containing the control PCR insert onto a LB–chloramphenicol (30 µg/ml) agar plate.[1]

       Note *The control PCR insert is a Pfu DNA polymerase-generated PCR product that contains a chloramphenicol-resistance gene. The control transformation reaction should be plated on LB-ampicillin agar plates and on LB–chloramphenicol agar plates to verify that the transformed colonies are also chloramphenicol-resistant.*

    c. Use a sterile spreader to plate 5 µl of the transformation reaction containing the pUC18 control plasmid into a 200-µl pool of NZY⁺ broth on an LB–ampicillin agar plate.

12. Incubate the plates overnight at 37°C.

13. Choose white colonies for examination (avoid colonies with a light blue appearance or colonies with a blue center). If necessary, patch white colonies onto a new LB–ampicillin agar plate containing X-gal and IPTG to verify the *Lac*⁻ phenotype.

    Note *Colonies containing inserts which were initially pure white may turn a light blue after ~2–5 days on the plate.*

Book 1003 pg 181 SSP [redacted]

Transformation Summary and Expected Results

Control transformation	Quantity of transformation	Plating quantity	Expected results: LB–ampicillin agar plates	Expected results: LB–chloramphenicol agar plates
pUC18 control plasmid	1 µl	5 µl	500 colonies (≥5 × 10⁸ cfu/mg DNA)	—
Control PCR insert	2 µl	50 µl	>100 colonies	>100 colonies*

* Growth of colonies on LB–chloramphenicol plates indicates successful insertion of the control PCR insert, which contains the chloramphenicol-resistance gene.

PCR Script Cloning Mega page 182

Setup Transformation for cloning PCR followed protocol steps 12 to 13.

Updated Download on IPTG/X-gal plates. Lori Clark had already updated @ 4C on [redacted]

To Page No. 182

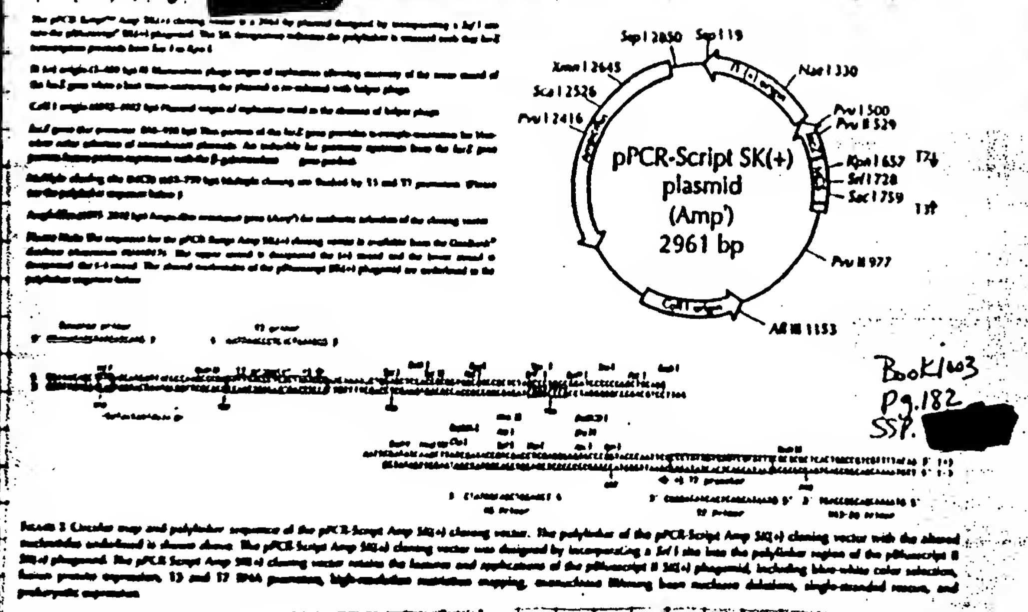
Witnessed & Understood by me, Lori M. Clark [redacted]

Invented by [redacted]

Recorded by [redacted] Date [redacted]

Project No. 414
Book No. 1003
TITLE pPCR-Script SK(+) Stratagene Map

From Page No. 181 [redacted]
178



Book 1003
Pg. 182
SSP. [redacted]

From page 178
John Ordubczak had transformed my ligation of pAVEh09. & pAVELYZ
[redacted] While I was on vacation in XBL2 cells. page 178
I picked colonies [redacted] 18 colonies - 3ml cultures Grew @ 30°C ON
Go to page 83
I examined my TCID 50s on Hep 3B + 293 cells of Homologous
Recombination Experiment page 177.
This is day 5 post infection

	Hep3B	293
SQ1-CVL	No rows show CPE	2 (Top 2 rows show CPE)
SQ1-P2	Top Row CPE	6 rows CPE
SQ1+pAVELYZ	2 rows CPE	7 rows CPE
SQ1 pAVELYZ + pCR	4 rows CPE	7 rows CPE.

I will officially read plates 10 days post infection

I submitted pBeloBAC plasmid stab to core to be bulked by Qiagen prep
This was plasmid Paul Hallenbeck got from Home Genome Systems.

T Page N

Witnessed & Understood by me,
Date [redacted]
Invented by
Recorded by
Date [redacted]

Project No. 414
Book No. 1060 TITLE Restriction Digests pAVEla09Lxi / Maxiprep of pCR-Script endostatin

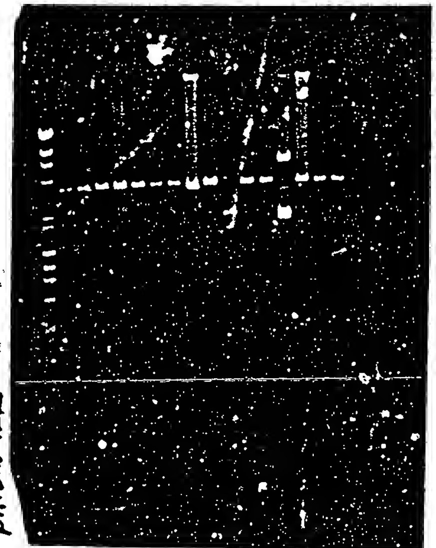
From Page No. 1 [redacted]

Ran check digests of pAVEla09Lx. on 1% agarose gel

Lanes

1	mwt λ HindIII ϕX Hae III	15	(15) BsiGI/BamHI
2	miniprep #1 BsiGI/BamHI.	16	16
3	2 "	17	17
4	3	18	18
5	4	19	Bk
6	5	20	Bk
7	6		
8	7		
9	9		
10	10		
11	11		
12	12		
13	13		
14	14		

Clone #14 is correct banding pattern. 2.017, 1.87 + 6.052 Kb.



Book 1060 pg. 4 [redacted]

Qiagen maxiprep of pCR-script human endostatin clone 2 page 2
Resuspended 250 ul H_2O Took OD reading 1:100 dilution

[illegible] [redacted]

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: Auto sample
Read average time: 0.50 sec

Units: ug/ul
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [N]

Book 1060 page 4
SSP. [redacted]

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ul	Nucleic Acid ug/ul
1	0.0005	0.0002	2.3478	0.4259	0.0000	0.0013
2	1.4997	1.5216	0.9856	1.0146	0.0000	3.7493

1.4997 x 50 ug/ml x 100 = 7.5 mg/ml

Set up check digests for endostatin

1ul DNA	1ul DNA	1ul DNA
2ul Buffer 1	2ul Buffer 2	2ul Buffer 1
2ul 10x BSA	0.75ul HindIII	1ul Xma I
0.75ul SacI	0.75ul StuI	16ul H_2O
0.75ul KpnI	15.5ul H_2O	20ul
13.5ul H_2O		

Incubate 37°C ON

To Page No.____

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

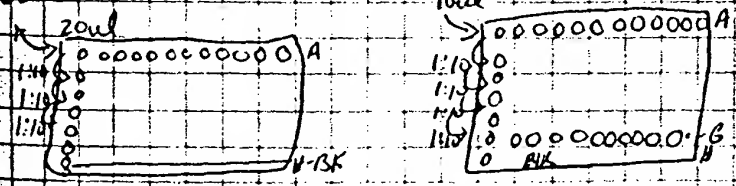
From Page No. 6

[redacted] from Wildtype Adenovirus

Set up transformation of pCR-Script human cerebostatin clone 2
Diluted miniprep DNA #2 → 1:100. Used 1ul in transformation.
1ul clone 2 + 100ul PH.5α cells.
30min on ice 42°C-45sec - 2min on ice.
Added 900ul SOC shaked @ 37°C 1hr
Plated 15ul + 40ul LB plates + 0.1ug/ml Ampicillin
Re also restreaked the clone 2 liquid cultures. Grew O/N @ 37°C

Setup limiting dilutions for cleaning up SQ1-P2 virus (AvnBglx virus) away from wildtype adenovirus. Plated 3T6 cells on [redacted] page 5
Need 10 particles/cell in each well of 96 well plate.
SQ1-P2 (TCH55) Virus was 1.92×10^{12} particles/ml
10 particles/cell × 2×10^4 cells = 2×10^5 particles
2×10^5 particles/well × $\frac{1 \text{ well}}{0.02 \text{ ml}}$ = 1×10^7 particles/ml Needed for infection
1×10^7 particles/ml × 1ml = 1×10^7 particles
Made 2 (1:100 dilutions) and then took 52ul into pool → 1:100
Made 1:100 dilution 52ul virus / 5000ul media (1.92 × 10^10 particles/ml) 10ul / 1000ul (1.92 × 10^8 particles/ml) → Took 52ul / 1000ul
for 1×10^7 particles.

Plated 20ul into top row of 96 well plate made 1:10 dilutions down through row G.
Plated 10ul into top row of 96 well plate made 1:10 dilutions down through row G



The dilutions will titer down to 1 particle/cells

To Page No.

Witnessed & Understood by me, [redacted] Date [redacted] Invented by Rec'd by Date [redacted]

Project No. 414
Book No. 1060 TITLE Cloning Bulk Preps pAVE1a09Lxi, Midipreps PCR-

From Page No. [redacted] Script Numerical Identification/Ratio of

Took the expansion plate of clone 2 PCR-Script human endothelin page 7. Southern blot picked one colony grew up in LB + 0.1 g/ml ampicillin @ 37°C

Prepared bulk cesium preps on pAVE1a09Lxi clone 14 via procedure page 33 Book 823 through cesium banding

Made 3 dpcm/stocks of pAVE1a09Lxi clone 14 (2 ml cubitainer + 2 ml SDS glycerol) store @ -70°C

Hybridized Southern blot BsrgI/BglII from Book 1003 page 159, 161, 179 used gap DNA probe from Loi Clarke.

Prehybridized blot - hybridization buffer + 100 ul Salmon Sperm 10 mg/ml boiled for 5 min. Hybridized approx 4 hr.
Added 15 uCi/g probe 68 ul of 0.22 uCi/ul. Probed O/N @ 68°C.

Calculated band ratios of Southern Blot BsrgI/BglII. This was probed from Book 1003 p. 179 with DNA which would identify plasmid input virus and new recombined virus AVE1LX2. The probe on page 149 Book 1003 was a 2.5 Kb fragment EcoHI/XhoI pAVNBglX.

Need to analyze 1 more lane + average; however it appears that the original SQ1-virus band is about 3.6-fold higher than the AVE1LX2 virus - the newly recombined virus

I will reanalyze blots tomorrow [redacted]

-1021

Book 1060 page 8 SSP [redacted]

	Volume		
RECT- 1	4871.52	3518	- SQ1 virus
RECT- 2	2311.41	- 957.78	AVE1LX2 virus
RECT- 3	1353.63	- Background	

} 3.7 fold difference between SQ1 & AVE1LX2 virus

To Page No. 9

Witnessed & Understood by me, Lisa M Clarke Date [redacted]

Invented by

Record d by [redacted] Date [redacted]

TITLE BM40 Signal Peptide for Human Endostatin

Project No. 414

Book No. 1060

From Page No. [redacted]

Ordered BM40 signal peptide oligos to add onto human endostatin PCR product. Michele Kaloss said that once oligos go above 100 bases that the fidelity of adding bases onto oligo may be altered, therefore giving wrong sequence. I went ahead and made 118 & 125-mer oligo. I will sequence after cloning.

Oligo Request Form

Name: Sandrina Phipps	Project Code: 414	Date: [redacted]
Length of Oligo: 118		
Purification: ☐ Unpurified ☒ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*		
Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)		

Sequence written 5' to 3' in triplets:

(HindIII) (BM40 signal)

GCC [AAG CTT] CCA TGA GGG CCT GGA TCT TCT
TTC TCC TTT GCC TGG CCG GGA GGG CTC TGG
CAG CCC CTC AGC AAG AAG CGC TCG CTC ACA (human endostatin)
GCC ACC GCG ACT TCC AGC CGG TGC TCC A

Total #G 32 #A 18 #T 24 #C 44

Book 1060 pg. 37 SSP [redacted]

Purpose: Cloning of BM 40 signal oligo into human endostatin plasmid. Approx 100 bases of BM40 signal peptide + human endostatin sequence 117 to 147 of pcrhend1 plasmid. Sense oligo has SexA1 restriction site at 3'end.

Name: Sandrina Phipps	Project Code: 414	Date: [redacted]
Length of Oligo: 123		
Purification: ☐ Unpurified ☒ OPC (oligo purification cartridge) *Note: oligos ≥ 40 bases will be OPC purified*		
Resuspension: ☒ H_2O ☐ TE ☐ None (return as dry pellets)		

Sequence written 5' to 3' in triplets:

CCA GGT GGA GCA CCG GCT GGA AGT CGC GGT
GGC TGT GAG CGA GCG CTT CTT GCT GAG GGG
CTG CCA GAG CCC TCC CGG CCA GGC AAA GGA
GAA AGA AGA TCC AGG CCC TCA TGG AAG CTT GGC

Total #G 46 #A 25 #T 18 #C 34

Book 1060 pg. 37 SSP [redacted]

Purpose: Clone the BM40 signal peptide onto human endostatin PCR product pcrhend1. The BM signal peptide is approx 100 bases followed by human endostatin sequence154 to 116 of pcrhend1 seq. Antisense oligo.

```
Molecule Name:      pcrhend1               1750 bps DNA Circular
Sequence Printed:   1-3750 (Full)          Date Printed [redacted]
Description:        Ligation of Fragment 1 and pCR-ScrptSK+

  1  cagatgacat cctggccagc ccccctcgcc tgcccgagcc ccagccctac
      Q  M  T  S  W  P  A  F  L  A  C  P  S  P  S  P
       R  -  H  P  G  Q  P  P  S  P  A  R  A  P  A  L
        D  D  I  L  A  S  P  P  R  L  P  E  P  Q  P  Y

 51  cccggagccc cgcaccacag ctcctacgtg cacctgcggc cggcacgacc
      T  P  E  P  R  T  T  A  P  T  C  T  C  G  R  H  D
       P  R  S  P  A  P  Q  L  L  R  A  P  A  A  G  T  T
        P  G  A  P  H  H  S  S  Y  V  H  L  R  P  A  R

101  cacaagccca cccgc[illegible]
      P  Q  A  H  P  [illegible]  R  C  S  T
       H  K  P  T  R  Q  P  P  R  L  P  A  G  A  P
        P  T  S  P  P  A  H  S  H  R  D  F  Q  P  V  L  H

151  tggttgcgct caacagcccc ctgtcaggcg gcatgcgggg catccgcggg
      W  L  R  S  T  A  P  C  Q  A  A  C  G  A  S  A
       P  G  C  A  Q  Q  P  P  V  R  R  H  A  G  H  P  R
        L  V  A  L  N  S  P  L  S  G  G  M  R  G  I  R  G

201  gccgacttcc agtgcttcca gcaggcgcgg gccgtggggc tggcgggcac
      G  P  T  S  S  A  S  S  R  R  G  P  W  G  W  R  A
       G  R  L  P  V  L  P  A  G  A  G  R  G  A  G  G  H
        A  D  F  Q  C  F  Q  Q  A  R  A  V  G  L  A  G
```

(human endostatin → primer) (SexA1)

Book 1060 Page 37 SSP [redacted]

The endostatin sequence highlighted in pink was added to BM40 signal peptide. The oligo goes through SexA1 site.

To Page No.

Witnessed & Understood by me, Lois M. Clark Date [redacted]

Invented by Sandrina Phipps Date [redacted]

Recorded by

Project No. 414
Book No. 1060 TITLE RT-PCR Canine Endostatin

From Page No. [redacted]

Diluted Canine Endostatin oligos to do RT PCR primer sequences are on Book 1003 pages 130-131 Sequence on page 115

Dilution 20uM / 500ul

2230 - 3.9mg/ml $\frac{3900}{0.33 \times 22\text{-mer}} = \frac{3900}{7.26} = 537uM$ 18.6ul oligo / 500ul

2231 - 3.1mg/ml 21-mer $\frac{3100}{6.93} = 447uM$ 22.37ul oligo / 500ul

2232 4.2mg/ml 24-mer $\frac{4200}{792} = 530uM$ 18.9ul oligo / 500ul

4ul 25mM MgCl2
8ul 10mM dNTPs
2ul PCR Buffer II
2.5ul H2O
1ul RNAse Inhibitor
1ul Random Hexamer or Oligo dT or 2231
0.5ul Canine RNA (22ug total 4.4ug/ul)
1.0ul RT (murine Moloney)

10 min @ room temp
15 min @ 42°C
5 min @ 99°C
Hold 4°C

EtoH ppt 2ul 3M NaOAc + 300ul EtoH Freeze -70°C 15 min.
Centrifuge 30 min. Wash X 70% EtoH.
Resuspend DNA 57ul H2O Set up PCR with PFU enzyme

57ul DNA
0.5ul 2231 (10uM final)
0.5ul 2232 (10uM final)
10ul PFU Buffer 10X
30ul dNTPs (2.5mM each)
98ul

Hot start add 2ul a 5 units ~~1000 units~~ of PFU
SSP.

3 min @ 95°C
3 min @ 80°C Add PFU
35 Cycles 1 min @ 95°C
1 min @ 55°C
3 min @ 72°C
Extend 15 min @ 72°C
Hold @ 4°C

Showed Theresa migration assay slides & discussed procedures

To Page No. 39

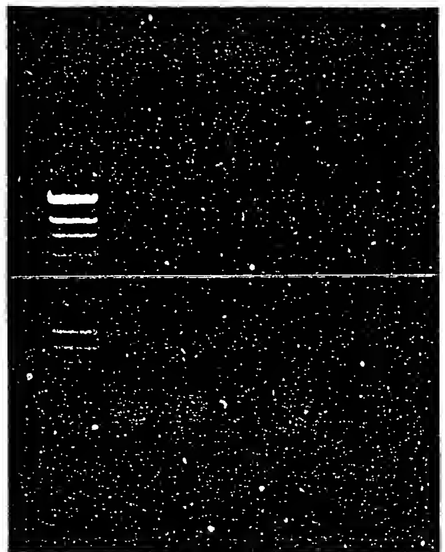
Witnessed & Understood by me, [signature] Date [redacted]
Invented by Date [redacted]
Recorded by [signature]

TITLE Canine RT PCR / Cloning of Human Endostatin with — Project No. 414 — Book No. 1060 — 39

From Page No. 38 [redacted] BM40 Signal Peptide

Ran endostatin PCR for Canine on gel
Lanes
1 mw+ λ Hind III ØX Hae III
2 oligo DT - 2231/2232 90ul
3 Random Hexamer - 2231/2232 90ul
4 Blk
5 2231 - 2231/2232 90ul
6 Blk

I cut out bands between 600 + 800bp region, will try to reamplify this



Canine RT PCR

Book 1060 ss [redacted] page 39

Page 42 (has)
Cloning scheme for BM40 Signal & human endostatin PCR
Digested pCRhend1 - SexA1 - because cuts in different buffer from HindIII.
This will be used to Clone BM40 oligos onto human endostatin PCR product
(Clone 2)

5ul pCRhend1 (clone 2) [redacted]
238ug/ul
2ul BSA 10X
2ul Buffer 3
1.5ul SexA1 (4u/ul)
9.5ul H2O
20ul

Incubate @ 37°C t_0 = 11:00
t_F = 1:00

EtOH ppt digest 2ul 3M NaOAC + 300ul EtOH
Put @ -70C 30min. Centrifuged resuspended in 15ul H_2O

go to page 40

I received BM40 signal peptide oligos Sequence page 37
Annealed oligos together so I can cut with HindIII to clone into HindIII / SexA1 site of pCRhend1

5.45ul oligo 2732 (6ug) 1.1ug/ul
4.6ul oligo 2733 (6ug) 1.3ug/ul
1.5ul Buffer 2
3.45ul H2O
15ul

Incubate PCR machine under DA33 program
Refer to Book ~~1063~~ SSP 893 page 19

To Page No. ~~42~~ 40

Witnessed & Understood by me, [signature] Date [redacted] Invented by — Recorded by [signature] Date [redacted]

Project No. 414
Book No. 1060 TITLE Cloning of BM40 Signal Peptide onto Human Endostatin

From Page No 39 [redacted] PCR

pCRhend 1
15ul H_2O/DNA
2ul Buffer 2
1.5ul HindIII (20u/ul)
1.5ul H_2O
20ul

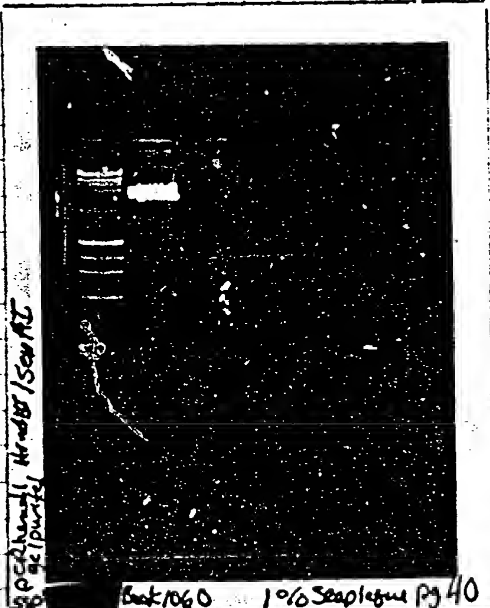
Oligo 2732
2733

15ul Oligo DNA
0.5ul Buffer 2
1.5ul HindIII
3ul H_2O

Incubate @ 37°C 2H.

Gel purify pCRhend 1 (HindIII/SexAI) to isolate 3.6Kb fragment.

Passed oligo through millipore micro enzyme extremeval column. Washed 5ul of column. EtOH ppt. Resuspended 10ul (0.6ug/ul)



I isolated band marked with arrow.
Did Promega 1U DNA cleanup kit.

Set up ligation
4.25ul oligo (annealed ~2.88ug SexAI/HindIII)
8.0ul PCR script hend 1 / SexAI/HindIII
2.0ul Ligase buffer
4.75ul H_2O
20ul Incubated @ 15° ON.

Need to run check gel to make sure Gel purified fragment is clean.

Collected (SSP)
I collected AVE1a 09Lxi virus CVL amplified 9 days on 293 cells ~~SF+ ce~~ upon original 3 plasmid transfection. I did not see CPE.

I obtained Hep 3B cells transfected with CSIRo#100 confirmed by Ke Wang with ~~lacZ~~ X-gal. Followed procedure Book 893 page 58. Left X-gal on ON.
Ke Wang did luciferase assay. I was staining for blue cell #

To Page No.

Witnessed & Understood by me, [signature] Date [redacted] Invented by Date [redacted]
Recorded by [signature]

TITLE BM 40 Signal Peptide Oligos

Project No. 414
Book No. 1060

From Page No. [redacted]

Applied Biosystems

Synthesis Order - 2733.898 [illegible]

[illegible] AM [redacted]
1948Control 2.2

Customer Name:
sp

Customer Address:

Book 1060
page 41
SSP [redacted]

Telephone Number: Fax Number:
PO Reference: Account Number:
Entry date: [redacted]
Comments:

1.3mg/ml

Run Protocol: SynPur 4.20
Post Synthesis: Purify Oligo
Sequence Name: 2733.898
Sequence:
5' CCA GGT GTA GCA [illegible] GCT GGA AGT [illegible] GGC TGT [illegible] CCA GCG [illegible] CTT GCT GAG GGG CTG
CCA GAG CCC TCC CGG CCA GGC AAA GGA GAA AGA AGA TCC AGG [illegible] TCA TGG AAG CTT GGC 3' SPW
Sequence Length: [illegible]
Base Composition:
A:025 G:046 [illegible]

Applied Biosystems

9:03 23 AM [redacted]
1948Control 2.2

Synthesis Order - 2732.898

Customer Name:
sp

Customer Address:

Book 1060
page 41
SSP [redacted]

Telephone Number: Fax Number:
PO Reference: Account Number:
Entry date: [redacted]
Comments:

1.1mg/ml

Run Protocol: SynPur 4.20
Post Synthesis: Purify Oligo
Sequence Name: 2732.899
Sequence:
5' GCC AAG CTT CCA TGA GGG CCT GGA TCT TCT TTC TCC TTT GCC TGG CCG GGA GGG CTC TGG CAG
CCC CTC AGC AAG AAG CGC TCG CTC ACA GCC ACC GCG ACT [illegible] AGC CGG TGC TCC A 3' SPW
Sequence Length: 118
Base Composition:
A:018 G:032 C:044 T:024 5:000 6:000 7:000 8:000

Request form for these oligos on page 37 and partial sequence of endostatin used.

Plated S8 cells to amplify AVE1a05Lxi virus and replated 14 T_{150} S8 cells → 1:20 to make roller bottles next week.
Passage 20

110 } 99.4x10^4 cells/ml   Need 1.33x10^5 cells/ml x 24ml = 3.2x10^6 cells / 99.4x10^4 cells/ml
89 }

Need 3.2ml + 20.8ml of Media + 72ul Dexamethasone (10uM) 0.3 final

Need 4x10^5 cells/well

Plated 1 6well plate 3ml/well.

To Page No.

Witnessed & Understood by me, [signature] M Clarke | Date [redacted] | Invented by | Date [redacted]

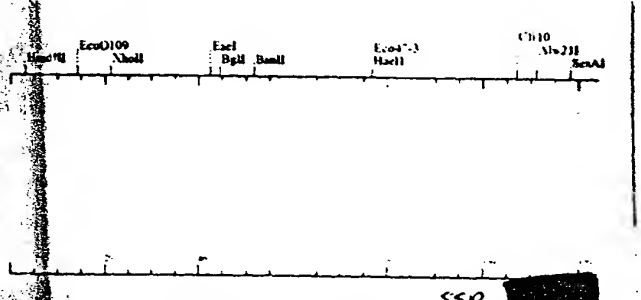
Recorded by Sandra Papp

Project No. 414
Book No. 1060 TITLE Cloning Scheme of BM40 Signal Peptide Addition

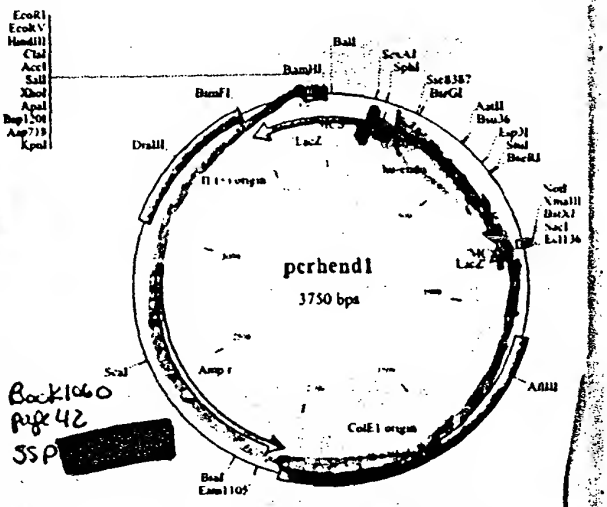
From Page No. 39

[redacted] to Human Endostatin PCR product.

We made BM40 signal peptide oligo with SexA1 overhang as it had already been cut.



SSP [redacted] Book 1060 page 42



Book 1060 page 42 SSP [redacted]

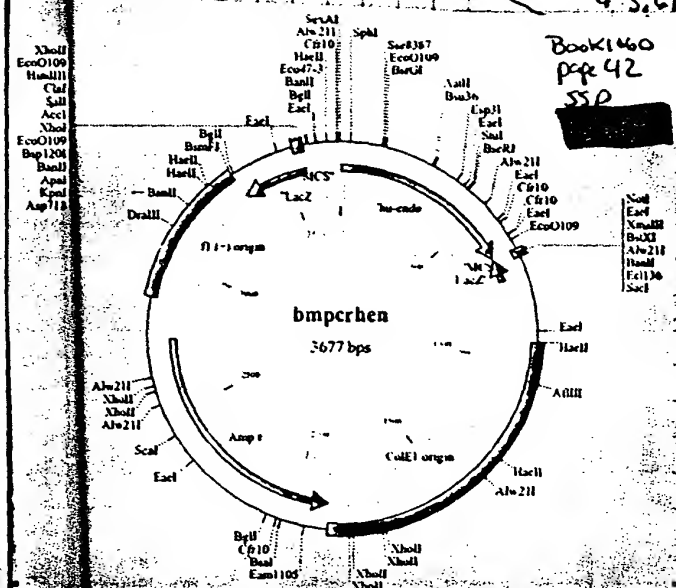
Cut pcrhend1 (HindIII)/SexA1)
Cut BM40signalpeptide HindIII/Hes. SexA1 site
HindIII

114bp

Ligate 114 bp & 3.6Kb fragment

HindIII/SexA1
Isolate 3.6Kb fragment

Book 1060 page 42 SSP [redacted]



Sequence of BM40 signal peptide is on page 41

To Page No.

Witnessed & Understood by me, [redacted] Date [redacted] Invented by [redacted] Date [redacted]

Recorded by [redacted]

TITLE Transformation of BM40 Signal peptide + Human Endostatin PCR

Project No. 414
Book No. 1060

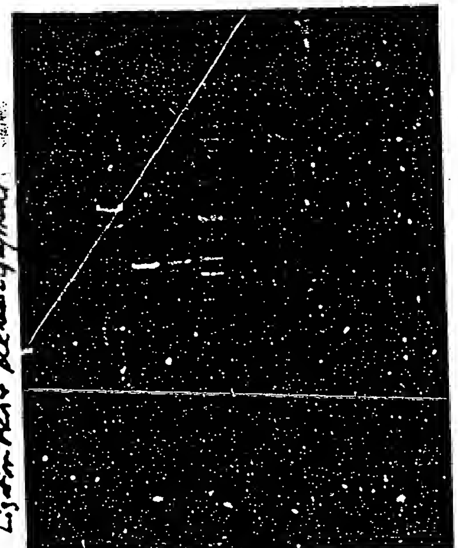
From Page No. [redacted]

Ran check gel to look @ ligation rxn of BM40 signal + human endostatin (bm pcr hen)
Ran gel purified pcR hand I (HindIII/SexAI) fragment 3.6Kb

Lanes
1. mwt λ HindIII φX HaeIII
2. 3.6Kb gel purified pcR hand I HindIII/SexAI 8ul
3. ligation rxn bm pcr hen 7ul
4. high mass marker 4ul
5-10 - Blank

It appears ligation occurred + pcR hand I fragment contains 1 band.
Freeze thawed AVE1a 09Lxi CVL 5X to infect 58 cells / dexamethasone.

NotI
XmaIII
BstXI
SacI
Ecl136



Ligation rxn + pcR hand I fragment

Book 1060 pg 43 [redacted] SSP

Set up transformation
4ul ligation rxn pcR hand I / BM40 signal + 100ul STBL2 cells
30 min on Ice
Heat shock 25sec @ 42°C
2min Ice
Add 900 ul to ligation rxn incubate @ 30°C 1½ Hr.
Plated 100 + 200ul on LB plates + ampicillin.
Grew ON @ 30°C.

Collected T75 CVLs of AVE1a 09Lxi (10 SSP) virus in 58 cells from page 34.
SQ1-P2 R5-10, 11, 3, 7. I threw out R5-8 because it lacked contaminated.
Collected SQ1-P2 R6-6 + 6-9. I threw away R6-8 because it looked contaminated.
I began amplifying the AVE1a09Lxi Virus CVL which had been grown with 3 plasmids in 293 cells on 6 well plates. From page 41.

Plated 0.5ml of CVL AVE1a09Lxi onto 4 wells of 6 well dish. Added 1.5 ul of 100uM dexamethasone to each well. Put @ 37°C 5% CO2

To Page No.

Witnessed & Understood by me.	Date	Invented by	Date
[signature]	[redacted]	Recorded by [signature]	[redacted]

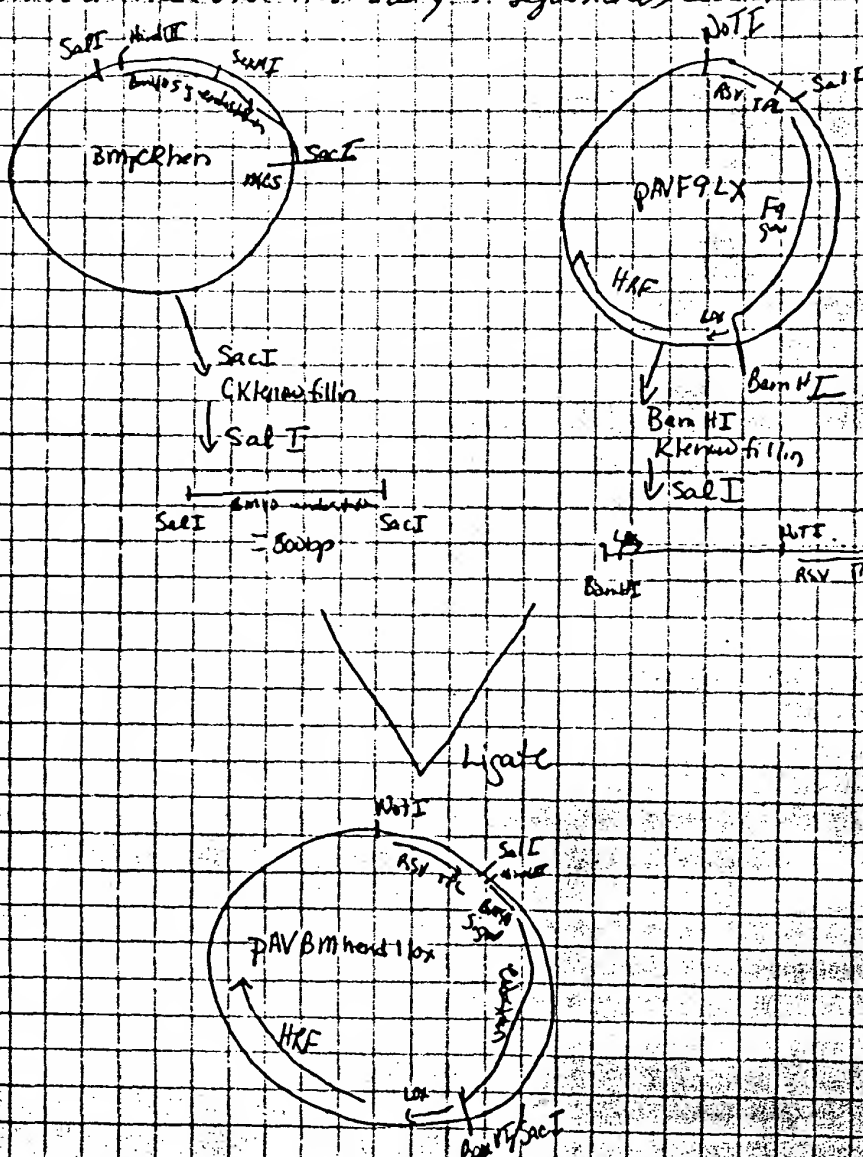
Project No. 414
Book No. 1060

TITLE Cloning Scheme for Human Endostatin with

From Page No. [redacted]

BM40 Signal peptide attached with Lox site

Set up digests. In BM40pcRhanI and F9LX plasmid. I am unsure if sequenced, however I will note and show results if sequencing is correct



Witness d & Understood by me, [redacted]

Date [redacted]

Invented by [redacted]

Recorded by [redacted]

Date [redacted]

Page No. 65

From Page No. 64 [redacted]

Viral genomic DNAs

Digest Bmp RhondI SacI
16ul 5ug 0.305ug/ul
2.5ul Buffer 1
2.5ul BSA 10x
1.5ul SacI
2.5ul H2O
25ul Incubate O/N @ 37°C

pAVF9Lx BamHI
5.7ul (10ug) 1.74ug/ul
2 ul BamHI Buffer
2ul BSA 10x
1ul BamHI
9.3ul H2O
20ul

[redacted]

1.0ul Klenow
25ul Bmp RhondI SacI
0.5ul Buffer 1
1.8ul 1mM dNTPs
2.7ul H2O
30ul

20ul pAVF9Lx BamHI
1.8ul 1mM dNTPs
1.0ul Buffer BamHI
1.0ul Klenow
6.2ul H2O
30ul SAP

Incubate @ 25°C 15min. - actually went approx 45min. Heat inactivated 15min @ 75°C EtOH precipitated go to page 66

OD Readings of SQ1-P2 genomic Viral DNAs

Sample	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ul	Nucleic Acid ug/ul	
	-0.0001	0.0001	-0.7965	-1.2555	0.0000	-0.0002	
	-0.0001	0.0002	-0.3981	-2.5117	0.0000	-0.0602	
	0.0001	-0.0001	-0.7963	-1.2553	0.0000	0.0002	
RS-7	0.0523	0.0374	1.3323	0.7179	0.0000	0.1301	
	0.0527	0.0379	1.3919	0.7185	0.0000	0.1317	.0525 x 50ug/ml x 50 = 131.25ug/ml = .131ug/ul
	0.0523	0.0377	1.4015	0.7135	0.0000	0.1319	
RS-11	0.0441	0.0229	1.9267	0.5190	0.0000	0.1103	
	0.0446	0.0228	1.9562	0.5112	0.0000	0.1115	.0445 x 50 x 50 ug/ml = 111.25ug/ml = 0.111ug/ul
	0.0449	0.0232	1.9375	0.5161	0.0000	0.1123	
RS-9	0.0216	0.0081	2.5864	0.3722	0.0000	0.0541	
	0.0227	0.0087	2.6081	0.3834	0.0000	0.0568	0.0225 x 50 x 50ug/ml = 56 ug/ml = .056ug/ul
	0.0232	0.0094	2.4748	0.4041	0.0000	0.0580	
RS3	0.0399	0.0162	2.4684	0.4051	0.0000	0.0998	
	0.0403	0.0165	2.4439	0.4082	0.0000	0.1008	.0402 x 50ug/ml x 50 = 100ug/ml = .1ug/ul
	0.0405	0.0166	2.4454	0.4059	0.0000	0.1012	
	0.0001	-0.0004	-0.1992	-5.0212	0.0000	0.0002	

Book 1060 page 65
SSP [redacted]

To Page No. 66

Witnessed & Understood by me, Lin M Clarke
Date [redacted]
Invented by
Recorded by Sandra Phipp
Date [redacted]

Project No. 414
Book N . 1060
TITLE Cloning of BM40 pCRhen into pAIF9Lx / Canine Endosh...

From Page No. 65

[redacted]

PCR

Setup digests with SalI for pAVF9Lx & pBM pCRhen I which have been cut with SacI, BamHI and Klenow filled in.

2ul SacI Buffer
2ul 10x BSA
1ul SalI
15ul DNA (Resuspended pellet 15ul H_2O)
20ul

Incubate ON @ 37°C

from page 63

Phenol/chloroform extracted AAV... viral genome ..., ... EtOH precipitated

Resuspended in 75ul H_2O.

Setup pCR nested with PCR rxns shown on page 57

10ul PCR rxn 2231/2230 , 2231/2232

2ul $MgCl_2$
5ul Buffer 2 PCR
0.5ul 2231
0.5ul 2230
1.0ul 10mM dNTPs
0.5ul Amplitaq Gold
30.5ul H_2O
50ul

12 min @ 94°C
35 cycles 1 min @ 95°C
1 min @ 56°C
1 min @ 72°C
Hold 10 min @ 72°C
Hold @ 4°C ON.

Split 293 cells passage 28 + 13 T150 1:20

Sandrine Phipps [redacted]

[redacted]

Ran 2% Seaplaque gel for PCR product 2231/2230

Lanes
1 mwt λHindIII φXHaeIII
2 2231/30 pCR - using 2231/30 primers 50ul
3 2231/32 pCR " " "
4 Low mass DNA marker 4ul
5-6 Blk

Ran 1% Seaplaque gel for DNA isolation

Lanes
1 mwt λHindIII φXHaeIII
2 pAVF9LX BamHI / SalI Klenow
3 BM40 pCRhen SacI / SalI Klenow
4 low mass DNA marker

To Page No. 67

Witnessed & Understood by me, Lori M. Clark
Date [redacted]
Invented by
Recorded by Sandrine Phipps
Date [redacted]

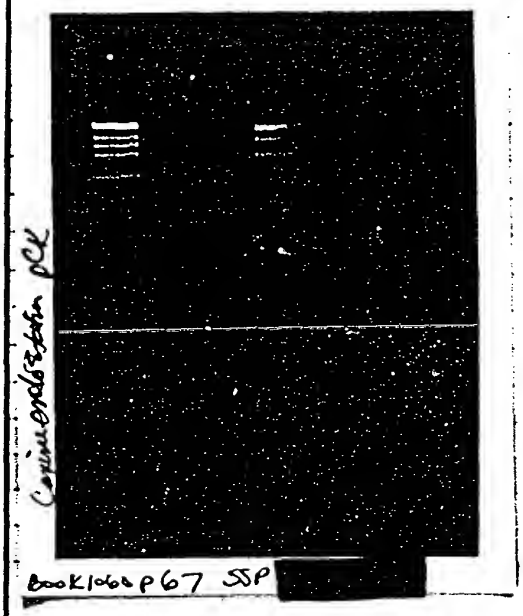
TITLE Cloning of bmy6 pCRhen into pAVF9Lx / Canine Endostatin

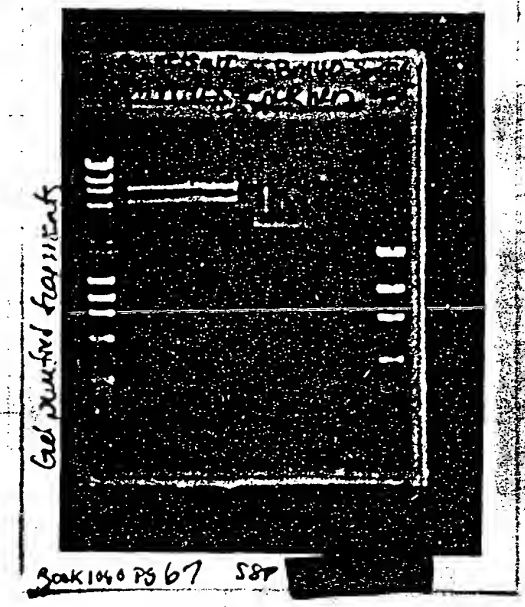
Project No. 414

Book No. 1060

From Page No. 66

[redacted] PCR





cloned arrowed fragments from pAVF9Lx+ BM40 pCRhen digested DNA.
The canine PCR did not work.
Planning to isolate poly A mRNA from dog liver.
Gel purified 6.9 + 0.800 kb gel fragments Promega PCR Clean up kit.
Ran 10ul on check gel on next page 68. The DNA is too dilute to see, however; I set up ligation anyway to transform

2ul ligase buffer
8ul pAVF9Lx (6.9 BamHI/SalI)
10ul BM4 pCRhen (0.8 Kb SacI/SalI)
1ul ligase
21 ul
Incubate ON @ 15°C

To Page No. 68

Witnessed & Understood by me,	Date [redacted]	Invented by	Date [redacted]
		Recorded by	

Project No. 414
Book No. 1060
TITLE TCID50 Titer SQ1-P2, AVE1a10Lxi, Production of SQ1-P2 R5-7

From Page No. 92

[redacted]

From page 92
Processed the SQ1-P2 R5-7 virus CVL from 8 roller bottle prep. as described pg. 20 Book 1003. Did 1.25/1.4 mg/ml CsCl-SW28 spin and ON spin in NVT65 @ 60,000 K in 1.33 mg/ml CsCl

Processed Hep 3B cells - trypsinized, + washed + put into 1% formaldehyde in PBS for Jane Hines negative controls for Kexon. Split Hep 3B 1:10 passage 52

Split 1 plate of S8 cells for transfection of AV3 + ~~AV~~ HP human endostatin virus. I forgot to linearize plasmids so therefore, I needed to plate new cells. Gave S8 5 plated on [redacted] for Lori Clarke. (Passage 17)

136 } 132 x 10⁴ cells/ml   I need 4 x 10⁵ cells/ml x 30 ml = 1.2 x 10⁷ cells
128 }   / 132 x 10⁴ cells/ml
                                                       = Need 2.3 ml + 90 ul dex...

Linearized SQ3, SQ4 plasmid + pBMhvd1X for 3 + 4 5 way transfections

SQ4 (4.3 ug/ul) 15 ug	3.5 ul	SQ3 (2 ug/ul) 20 ug	10 ul	pBMhvd1X (3.1 ug/ul) 30 ug	9.68 ul
	2.0 ul Buffer H		2.0 ul Buffer H		2 ul Buffer
H_2O	12.5 ul		6.0 ul H_2O		2 ul BSA
	2.0 ul Cla I 40u/ul		2.0 ul Cla I 40u/ul		4.32 ul H_2O
	20 ul		20 ul		2.0 ul Not I
					20 ul

Incubate @ 37°C ON
Read TCID50 for titer SQ1-P2 R5-3 + AVE1a10Lxi - 1 roller bottle preps

Book 1060
pg. 94
SSP
[redacted]

> 3.45 x 10⁹ pfu/ml
20 ul/well
Sample: S8 cells SQ1-P2 R5-3

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D	+	+	+	+	+	+	+	+	+	+	+	+
E	+	+	+	+	+	+	+	+	+	+	+	+
F	+	+	+	+	+	+	+	+	+	+	+	+
G	+	+	+	−	−	−	+	−	−	+	+	+
H												

Dilution: 1:100 dilution from 10⁻¹¹ particles/ml to 10⁻⁹ particles/ml

Sample: AVE1a10Lxi Virus S8 cell 1:100 dilution
20 ul/well [redacted]

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E	+	+	+	+	+	+	+	−	+	+	+	+
F	+	−	−	−	−	−	−	−	−	+	−	−
G	−	−	−	−	−	−	−	−	−	−	−	−
H												

Sept. 29, 1998
Book 1060
pg. 94
SSP
1.23 x 10⁸ pfu/ml

To Page No. 95

Witnessed & Understood by me, Lori M Clarke
Date [redacted]
Invented by
Recorded by Sandra Phipps
Date [redacted]

From Page No. 94 [redacted] Plasmid System

Pulled virus band of SQ1-PZRS-7 total volume 2.8ml added 2.8ml/9ml = 0.311 ml of glycerol. Dialyzed in cassette through 4 changes of HBS Buffer 1x + glycerol @ 4°C - 4 liters. Last dialysis went O/N.

Set up 3 plasmid transfections of SQ3 + SQ4 with pBMHendIx plasmid.

pSQ3 (ClaI)	1ug	1ul	} +6ul plus reagent +10ul Optimem media
pCre	0.3ug	1ul	
pBMhendIx (NotI)	0.5ug (1.5ug/ul)	0.5ul	

Set up 2 rxns → put on 58 cells.

pSQ4/ClaI	1ug	1.33ul (0.75ug/ul)	} +6ul plus reagent +100ul Optimem media
pCre	0.3ug	1ul (0.3ug/ul)	
pBMhendIx part I	0.5ug	0.5ul (1.5ug/ul)	

Set up 2 rxns → put on clone 54 cells.
Incubate 15 min @ room temp.
Add 4ul lipofectamine + 100ul Optimem. Made 4 1/2 rxns 45ul media + 18ul lipofectamine

Incubate @ room temp. for 15 min.
Add 2.8ml of Optimem media to cells overlay 200ul of DNA/lipofectamine complex to cells.
Incubated @ 37° 5% CO2 4 1/2 Hr. Removed transfection rxn
Added 4ml of R. Ch Hes + 5% FBS to clone 54 + 58 cells.

Set up 5 way transfection to SQ4 to make AV4 virus on 293 cells in 35mm wells (6 well plate)

pC-Cre (Cre)	1ug	(2ul)	or	1ug	(2ul)
pBRES-E4 (E4)	0.5ug	(0.5ul)		0.5ug	0.5ul
pCE2a (E2a)	0.5ug	(0.5ul)		0.5ug	0.5ul
pSQ4cutW/ClaI	1.5ug	(2.0ul)		1.0ug	1.33ul
pBMhendIx/NotI - shuttle plasmid	3.75ug	(2.5ul)		2.5ug	1.67ul
Use Calcium phosphate kit	H_2O	102ul			103.5ul
	$CaCl_2$	15.5ul			15.5ul
		125ul			125ul

To Page No. 96

Witnessed & Understood by me. Lisa M Clarke Date [redacted]

Invented by [redacted]

Recorded by [redacted]

Fr m Page No. 95

[redacted] Production

Bubbled DNA+Hg CaCl2 complex into 2x Hepes while Vortexing.
Incubate @ room temp 30 min.
Overlay 250ul onto 2 wells of 6 well plate
w/ changed media Richters + 10% FBS before dosing transfection - approx 3 Hr.
Incubated 37°C 5% CO2

Setup TCID50 titer plate for SQI-P2 R5-3 because original TCID50 titered off the plate.

Took the 9/18 1:100 dilution Made another 1:10 dilution, then used this tube to make 10-fold dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} input onto 96 well plate

10^{-1}	Row B	10^{-5} Row E	To Calculate TCID50 the original starting dilution
10^{-2}	Row B	10^{-6} Row F	Will be 1:10,000.
10^{-3}	Row C	10^{-7} Row G	I put 200ul in each well
10^{-4}	Row D	Blank control Row H	

[redacted]

[redacted]

Aliquoted SQI-P2 R5-7 SSP4-98 VIRUS
22 vials 20ul
20 vials 50ul
3 vials 100ul
Virus

Prepared Particle concentrations
10ul virus incubate @ 56°C with shaking 10 min.
90ul viral concentration buffer incubate @ room temp 10 min.
Took 260nm OD. Made 1:10 dilutions 1 OD = 1.12×10^{17} particles/ml
1. 0.335 $0.335 \times 10 \times 1.12 \times 10^{17}$ particles/ml = 3.752×10^{18} particles/ml
2. 0.180 $0.180 \times 10 \times 1.12 \times 10^{17}$ particles/ml = 2.016×10^{18} particles/ml
3. 0.178 $0.178 \times 10 \times 1.12 \times 10^{17}$ particles/ml = 1.99×10^{18} particles/ml
I think I must have used 25ul in #3 tube it is approx double
Samples 2+3
Averaged # 2+3 together for particle titer = 2.025×10^{18} particles/ml
Made 1:100 dilutions of other 10ul to titer virus in Richita's + 5% FBS

To Page No. 97

Witnessed & Understood by me. Lisa M. Clark Date [redacted]
Invented by [redacted] Date [redacted]
Recorded by [redacted]

TITLE AVE1a09Lxi particle titer / Amplification

Project No. 414
Book No. 1060

From Page No. [redacted] AV3 human endostatin Virus

Collected 2nd amplified CVL from AV3 + human endostatin on 58 cells 7 days post infection. Centrifuged & removed media to 3 ml. Resuspended CVL in 3 ml media.

This was 2nd amplification of SQ3 + human endostatin which was transfected in 88 cells with lipofectamine plus. Froze @ -70°C.

Set up particle OD readings for AVE1a09Lxi Lot # SSP5-98

Took 20ul of 20ul aliquot vial

(2) 20ul out of 50ul aliquot vial

Added 80ul Viral concentration buffer to each tube (Book 1003 pg. 25)

Incubate @ 56°C 10 min with Shaking.

Incubate @ RT. 10 min. Read 260nm OD. Dilution made was 1:5

Read ODs in duplicate

BECKMAN DU-640 Date [redacted]

AVE1a09Lxi

Book 1060 page 119

S. Phipps [redacted]

Nucleic Acid
Read Samples Method Save Clear Print Quit

Results file: A:\NUCL_RES Method name: A:\DEFAULT

Assay type: General Ratio and Concentration Units: ug/ml
Formula setup: VIEW Background Correction: [No]
Sampling device: None Concentration: [Yes]
Read average time: 0.50 sec Peak Pick: [No]

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml	
1	0.0743	0.0655	1.1348	0.8812	0.0000	0.1857	0.076 x 5 x 1.12x10^12 particle/ml = 4.256 x 10^11 particle/ml
2	0.0774	0.0674	1.1484	0.8708	0.0000	0.1934	
3	0.0723	0.0637	1.1341	0.8818	0.0000	0.1806	0.073 x 5 x 1.12x10^12 particle/ml = 4.088 x 10^11 particle/ml
4	0.0734	0.0644	1.1402	0.8766	0.0000	0.1836	
5	0.0728	0.0632	1.1413	0.8762	0.0000	0.1820	0.073 x 5 x 1.12x10^12 particle/ml = 4.088 x 10^11 particle/ml
6	0.0741	0.0646	1.1462	0.8725	0.0000	0.1852	

Average 4.14 x 10^11 particles/ml

Particles/ml 4.14 x 10^11 particles/ml
AVE1a09Lxi SSP5-98

To Page No.

Witnessed & Understood by me, Kim M Clark Date [redacted]

Invented by

Recorded by Sandra Phipps Date [redacted]

From Page No. 120

[redacted] of Human Endostatin.

Freeze thawed Homologous Recombination Hep3B amplified CVLS 4X as well as AV4-3 plasmid clone 54. CVL from 2nd amplification of 3 plasmid transfection collected CVL 7 days post infection. - freeze thawed 4.5X as well as AV3- human Endostatin 3rd Round amplification.

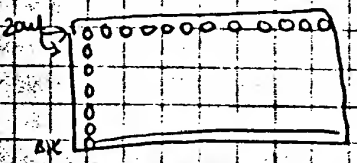
Put 2ml of AV3 human endostatin (2nd amplified CVL) from 3 plasmid transfection on 5 Bculfs onto T75 1X10^7 Sf cells + dexamethasone 0.3uM this is 3rd amplification.

Collected AV4 - 2nd amplification CVL from 293 cell (5 plasmid transfection) which had been amplified on clone 54 cells.
Collected CVL/supt. 12mls for 1:67 up + 375up of DNA used.
Centrifuged - removed 9ml of supt stored @ 4°C for endostatin test and Resuspended 3ml Unto cells. Store @ -70°C Combined 3 wells of each together in 50ml conical tube.

Set up TCID50's of SQI-P2 R5:7 (20ul aliquot SSP4-98 Lot II
AVEla09Lxi (20ul aliquot SSP5-98-Lot II

Diluted SQI-P2 1:10,000 because original 1:100 titered off plates)
1:100 → 1:100 IMDM+5%FBS
10ul/990 → 10ul/990 → Made serial 10fold dilution 100ul/900 → 100ul/900
10^{-1} 10^{-4} 10^{-7} Put 20ul unto each well using combitip.
10^{-2} 10^{-5}
10^{-3} 10^{-6}

Diluted AVEla09Lxi 1:1000 in IMDM+5%FBS
1:100 → 1:10
10ul → 990 → 100ul/900 Made serial dilution 10^{1} → 10^{-7} 10ul → /90ul



Set up viral genomic DNA extractions of SQI-P2 R5-7+ AVEla09Lxi virus
20ul → Incubate @ 56C with shaking/approx 3-4H.
200ul TES → Phenol Chloroform extract / Chloroform isoamyl alcohol extract
5.72ul proteinase K → Ethanol ppt ON @ -20°C.
Vortex well

To Page No.

Witnessed & Understood by me, [redacted] Date [redacted] Invented by [redacted] Date [redacted]
Recorded by [redacted]

Project No. 414
Book N . 1060 TITLE Collection of Homologous Recombination CVLs / Collection

From Page No. 131 [redacted] of AV4 Human Endostatin CVLs

Plated 11 T_{150} 1:3 + 75ul dexamethasone to amplify AV3 human endostatin
Plated 5 T_{150} @ 1:10 to set up for roller bottles for making AV3 human endostatin

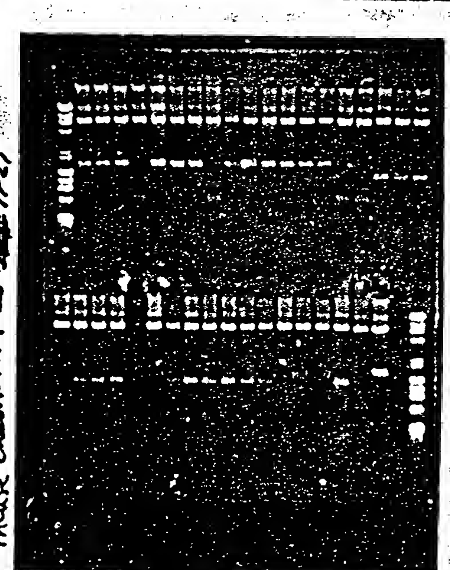
Collected CVLs of homologous recombination experiment page 126, 130.
I collected 1a,b, 5a,b, 6, 9b, 7a,b, 8a,b, and SQ1-P2 alone after day 6 postinfection. I need to reamplify 5-8 on Hep3B cells.
Snap froze dry ice, froze @ -70°C.

Collected AV4 clone 54 human endostatin 3rd amplification → 3 wells of 6 well plate CVL and supernatant. I collected cells + supt. Centrifuged 5min @ 1800RPM → removed 8ml of supt stored @ 4°C for human endostatin elisa testing. Froze remaining cells + supt for CVL @ -70°C

Collected AV4 human endostatin 3rd amplification made by 5 plasmid in 293 and passaged 2x on clone 54 cells. I had used 1.67 + 3.75ug of DNA. Collected 6 wells + pooled together for each DNA concentration. Centrifuged + removed 15ml of supt. Made CVL out of cells + remaining sup. Stored sup @ 4°C

Ran gel for pAVTPLi and LX clone gel
1% Agarose
Top gel
Lanes
1 - mwt λHindIII ϕXHaeIII
2 - 16 minipreps AscI/NheI
18-20 minipreps # 1-3 NotI

Bottom gel
Lanes
1-4 minipreps # 4-7 NotI
5 - BK
6-16 minipreps # 8-18 NotI
17-18 - minipreps # 17-18 NheI/AscI
19 - BK
20 - mwt λHindIII ϕXHaeIII



S.Priggs [redacted] Book 1060 p3

To Page No. 133

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

Project N . 414
Book No. 1060 TITLE Bulk Preps of AVTPLi BMHendLx and LX

From Page No. [redacted]

3rd Amplification of Homologous Recombination CVL

↓ clone 3 ↓ clone 1

Grew up 500 ml cultures of pAVTPLi BMhendLX and pAVTPLi endLX in Yet Broth + PO4 + 100 μg/ml ampicillin.
Grew ON @ 30°C for Bulk plasmid preps.

Freeze thawed AV3 human endostatin 3rd amp CVL and Homologous Recombination Supernatant CVLs from Hep3B cells 5X

Put 3rd amplification of Homologous Recombination CVLs onto Hep3B cells plated on page 131.

Plated 5a, b, 6a, b, 7, a, b, 8a, b and #1 original pAVFGLX 4ug + SQI-virus onto 2 6well plates.
Incubated ON @ 37°C 5% CO2 1ml on each well rocked ON
At 20 hr post infection will remove CVL wash 2X PBS and add 4ml DMEM + 10% FBS/well

5a	6a	7a		8a	Blank	← #1 original CVL from transfection infection
o	o	o		o	o	o
o	o	o		o	o	o
5b	6b	7b		8b		

↗ 2nd amplification CVL.

I should have used 1st amplification CVL for #1 after transfection infection 1a & b collected on 10/19/98 instead of original. I am reamplifying #1 because 2nd CVL has some CPE but it was next to wells highly infected with AVEGLX2 + SQI @ 4ug/well which had been harvested. We want to make sure this CPE was not caused from splash over or cross over from well to well while harvesting was done. t_0 = 12:30 pm

Setup 58 amplification of 2nd Round amplification of CVL from #1a, b, #2a, b #3a, b #4 a, b, SQI a+b

#1 → o o o o o o o o o o o o (1a 1b 2a 2b 3a 3b)
#2 → 4a 4b
#3
SQIa SQIb

I did duplicate wells of each CVL for PCR 1ml/well. On Friday I will denature cells & prepare for PCR

To Page No. 137

Witnessed & Understood by me, Date [redacted] Invented by Date [redacted]
Lin M Clark [redacted] Recorded by Sandra Phipps

Project No. 414
Book No. 1060 TITLE Infection of Various Cell Lines With AVE...

From Page No. 147 [redacted]

AVEa09Lxi

For HUVEC cells I only used 2x10^4 cells/well. I had raw dilutions for infection

10 particles/cell x 2x10^4 cells/well x 1 well/0.1ml = 2x10^6 particles/ml

2x10^6 particles/ml x 1ml = 2x10^6 particles - need 8.3ul Add1312 / 1ml
12.6ul Add1327 / 1ml
48.3ul AVEa09Lxi / 1ml
14ul AVEa10Lxi / 1ml

1. All wells were infected in duplicate.
2. Washed wells 1X PBS.
3. Added 0.1ml virus onto each well except for LS180 which gets 0.2ml of virus.
4. Infect @ 37°C and 5% CO_2 with rocking for 3 hr.
5. Washed wells 1X after removing virus. Then added ~~warming~~ fresh media appropriate for the cells 2ml/well.

It appears part of wells may have dried out. I need to infect with 0.2ml if this experiment is repeat.

Met with Mike Perry 1 1/2 hr - all hands meeting

From page 146

I infected three wells of AV4 human endostatin 3rd round amplified CVL of 6 well plate 0.4ml / well. This was only remaining CVL after contamination. I filtered through 0.2uM filter. Reamplified 4th time Clone 54 cells. (2.5ug)

Infected 4 wells of clone 54 cells (3.75ug DNA) AV4 human endostatin 3rd round amplified ~~CVL~~ CVL. ASP

Infected 1 well of clone 54 cells with AV4 human endostatin made 3 plasmid system in Clone 54 cells

Infected 8 roller bottles with AV3 human endostatin
30ml IMEM + 2% FBS + 0.3uM dexamethasone + 1ml of CVL / bottle
After 3 Hr added IMEM + 5% FBS up to 150ml.
Incubate @ 37°C 5% CO_2

To Page No.

Witnessed & Understood by me, [signature] Date [redacted]

Invented by

Recorded by [signature] Date [redacted]

Project No. 414
Book No. 1060 TITLE Xgal Staining of Transient Transfection 293T cells of pAVIPL, and Lx d BMhandLx

From Page No. 155 [redacted]

I X-gal stained the 4 plates transfected with pAVnBgLX.

Washed plates 1x PBS. Added formaldehyde, glutaldehyde fixative 5ml (10min @ 4°C)
Washed plates 1X PBS and added X-gal reaction mixture.
Stained @ 37°C 5% CO_2 for approx 4hrs
Staining procedure book 893 page 58.

Prepared AV3 BMhandLx (human endostatin cesium prep - 8 roller bottles) carried through to OD spin in NKT 6 5 Book 1025 pg. 19-21.

Split Chang cells P3 1:20 T_{150}
Split SaOS-2 cells - McCoy's + 15% FBS + P/S 1:8 in 3 T_{150} to freeze some cells down.

Split 58 cells p24 1:3 into 3 flasks.

Set up 20 293 96 well plates for TCID 50 of Homologous recombinant CVLS 1a - 8a, b.
293 passage 23
88 }
67 } 85x10⁴ cells/ml — Need 5x10⁴ cells/ml x 430ml = 2.15x10⁷ cells
100 } — Need 25.3 ml cells + 404.7 ml media
Plated 200ul/well → 1x10⁴ cells/well.

Plated a 6 well plate to set up transfection of AV4 human & mouse TP into endostatin again.

1x10⁵ cells/ml x 27ml = 2.7x10⁶ cells / 85x10⁴ cells/ml Need 3.2ml cells.
(Plated 20 96 well plates)

Split Hep 3B cells passage 60
44 }
38 } 41.33x10⁴ cells/ml Used all cells to try to plate close to 1x10⁴ cells/well
42 } in 96 well plates. Plated 19 plates.
30ml of cells x 41.33x10⁴ cells/ml = 1.24x10⁷ cells / 380ml = 3.3x10⁴ cells/ml
Plated 200ul of 3.3x10⁴ cells/ml for 6.5x10³ cells/well

To Page No. 159

Witnessed & Understood by me, [signature] Date [redacted]
Invented by
Recorded by [signature] Date [redacted]

TITLE Human Endostatin Elisa

Project No. 414

Book No. A60

From Page No. 156 [redacted]

The 293 cells transfected on page 151 - many of cells rounded up & came off plate (AV4 human mouse TPLinton endostatin); therefore I may repeat transfection

Sandra [signature] [redacted]

[redacted]

Pulled cesium band virus AV3 human endostatin processed on page 156. Measured band 2.15ml added 0.239ml glycerol 1:(2.15/9ml) Centrifuged, loaded in dialysis cassette Dialyzed in THP buffer 4X - 4 liters total @ least 1hr/liter.

Freeze thawed homologous recombination cells 5X

Set up human endostatin elisa

Samples were as followed (Supernatant from AV3+AV4 human endostatin)

1. Human TPLinton endo + nBg } Transient Transfection Supt in 293 cells
2. Human endo + nBg } [redacted] 72 h post transfection supt
3. Human TPL inton endo
4. Human endo endo
5. mouse endo
6. mouse TPLinton endo
7. mouse TPL inton nBg
8. mouse endo nBg
9. AV3 human endostatin (from 8 roller bottle prep
10. AV3 human endostatin Supt 3rd amp [redacted]
11. AV4 human endostatin supt Clone 54 2nd amp [redacted]
12. AV4 clone 54 (293 S plasmid) 3.75 μg 3rd amp [redacted] 6 day p.i.
13. AV4 Clone 54 (293 S plasmid 1.67 μg 3rd amp [redacted] 6 day p.i.
14. AV4 clone 54 (Clone 54 3rd round amp) [redacted] 6 day p.i.
15. AV4 Clone 54 (293 S plasmid) endostatin 2nd amp 1.67 μg DNA [redacted] 7 day p.i.
16. AV4 Clone 54 (293 S plasmid endostatin 2nd amp 3.75 μg DNA [redacted] 7 day p.i.

Layout of endostatin plate on page 168

Followed standard elisa protocol 50 ng/ml → 1.95 ng/ml detection. Protocol pages 105-106

To Page No. 158

Witnessed & Understood by me, [signature]

Date [redacted]

Invented by [signature]

Date [redacted]

Recorded by [signature]

Project No. 414
Book N . 1060 TITLE Endostatin Elisa

From Page N . 157 [redacted] Elisa Plate Format

Book 1060 page 158 S.Phipps [redacted]

	1	2	3	4	5	6	7	8	9	10
A	standard	1.95	#1 1:2	#5 1:2	#9 1:2	#13 1:2	#1 1:4	#5 1:4	#9 1:4	#13 1:4
B	standard	1.95	#1 1:2	#5 1:2	#9 1:2	#13 1:2	#1 1:4	#5 1:4	#9 1:4	#13 1:4
C	1.25	ODmax	#2 1:2	#6 1:2	#10 1:2	#14 1:2	#2 1:4	#6 1:4	#10 1:4	#14 1:4
D	1.25	ODmax	#2 1:2	#6 1:2	#10 1:2	#14 1:2	#2 1:4	#6 1:4	#10 1:4	#14 1:4
E	3.125 ~~7.81~~	1:2 media	#3 1:2	#7 1:2	#11 1:2	#15 1:2	#3 1:4	#7 1:4	#11 1:4	#15 1:4
F	3.125	1:2 media	#3 1:2	#7 1:2	#11 1:2	#15 1:2	#3 1:4	#7 1:4	#11 1:4	#15 1:4
G	7.81	1:4 media	#9 1:2	#8 1:2	#12 1:2	#16 1:2	#4 1:4	#8 1:4	#12 1:4	#16 1:4
H	7.81	1:4 media	#11 1:2	#5 1:2	#12 1:2	#16 1:2	#4 1:4	#5 1:4	#12 1:4	#16 1:4

Corresponding samples are given on page 157

[redacted] Book 1060 page 158 S. Phipps [redacted]

	1	2	3	4	5	6	7	8	9	10	11	12
A	[redacted]											
B												
C												
D												
E												
F												
G												
H												

To Page No. 159

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by [redacted] Date [redacted]

Recorded by [redacted]

TITLE Endostatin Elisa

Project No. 414

Book N 1060

From Page No. 158

[redacted]

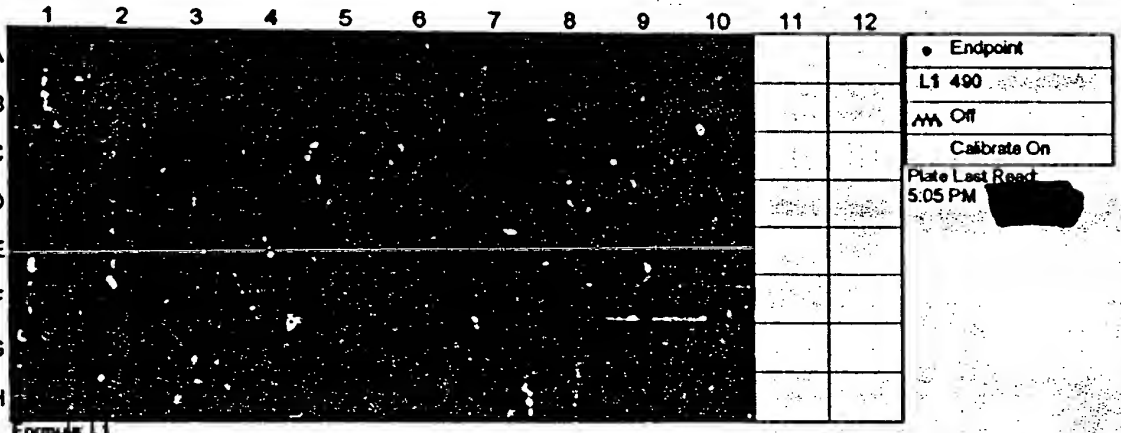
Book 1060 page 159

11/10/98

Experiment#1

[redacted] Sandra Phipps

Plate#1



Formula: L1

Data Mode: Absorbance

Book 1060 page 159

[redacted] Standards (ng/ml) S. Phipps

Sample	Concentration	BackCalcConc	Wells	Values	MeanValue	Std.Dev.	CV%
Std1	500.000	Range?	A1	0.188	0.200	0.017	8.5
		2194.026	B1	0.212			
Std2	125.000	106.785	C1	0.572	0.522	0.070	13.5
		143.302	D1	0.472			
Std3	31.250	27.646	E1	1.456	1.423	0.046	3.2
		29.730	F1	1.391			
Std4	7.810	8.838	G1	2.477	2.450	0.038	1.5
		9.459	H1	2.424			
Std5	1.950	2.729	A2	3.102	3.185	0.118	3.7
		1.279	B2	3.268			
Std6	0.000	Range?	C2	3.416	3.395	0.029	0.8
		0.263	D2	3.375			

Smallest standard value: 0.200

Largest standard value: 3.395

To Page No. 160

Witnessed & Understood by me, [redacted]

Date [redacted]

Invented by

Recorded by Sandra Phipps

Date [redacted]

Project No. 414
Book No. 1060 TITLE Human Exploitation Elisa

From Page No. 159 [redacted]

UnknownsDilution

Book 1060 page 160
S. Rupp

Sample	Wells	Values	R	Result	MeanResult	Std Dev.	CV%	Dilution	Adj Result
Un01	E2	3.329		0.723	0.723	0.000	0.0	2.0	1.446
	F2	3.437	R	Range?					
Un02	G2	3.365		0.370	0.868	0.704	81.1	4.0	3.471
	H2	3.258		1.366					
Un03	A3	1.370		30.454	28.174	3.224	11.4	2.0	56.349
	B3	1.515		25.895					
Un04	C3	2.211		12.186	13.385	1.696	12.7	2.0	26.770
	D3	2.050		14.584					
Un05	E3	1.852		18.040	17.818	0.314	1.8	2.0	35.635
	F3	1.875		17.596					
Un06	G3	0.796		66.751	69.915	4.475	6.4	2.0	139.830
	H3	0.746		73.079					
Un07	A4	3.206		1.825	1.338	0.690	51.6	2.0	2.675
	B4	3.316		0.850					
Un08	C4	3.358		0.444	0.796	0.497	62.5	2.0	1.591
	D4	3.283		1.147					
Un09	E4	3.341		0.609	1.725	1.578	91.5	2.0	3.450
	F4	3.089		2.841					
Un10	G4	3.324		0.770	0.635	0.192	30.2	2.0	1.269
	H4	3.352		0.499					
Un11	A5	0.281		421.473	377.655	61.969	16.4	2.0	755.309
	B5	0.306		333.836					
Un12	C5	0.412		180.290	172.435	11.110	6.4	2.0	344.869
	D5	0.434		164.579					
Un13	E5	3.229		1.625	1.163	0.654	56.2	2.0	2.326
	F5	3.331		0.700					
Un14	G5	3.382		0.185	0.661	0.674	101.9	2.0	1.323
	H5	3.284		1.138					
Un15	A6	3.341		0.613	0.613	0.000	0.0	2.0	1.226
	B6	3.414	R	Range?					
Un16	C6	3.242		1.507	0.848	0.932	110.0	2.0	1.695
	D6	3.381		0.188					
Un17	E6	3.328		0.730	0.638	0.129	20.3	2.0	1.276
	F6	3.347		0.547					
Un18	G6	3.219		1.715	3.031	1.861	61.4	2.0	6.063
	H6	2.920		4.347					
Un19	A7	2.161		12.900	11.724	1.663	14.2	4.0	46.897
	B7	2.335		10.549					
Un20	C7	2.685		6.602	6.333	0.380	6.0	4.0	25.332
	D7	2.738		6.064					
Un21	E7	2.672		6.730	7.377	0.914	12.4	4.0	29.506
	F7	2.550		8.023					
Un22	G7	1.419		28.806	29.212	0.575	2.0	4.0	116.849
	H7	1.394		29.619					
Un23	A8	3.209		1.804	1.451	0.498	34.3	4.0	5.806
	B8	3.288		1.099					
Un24	C8	3.307		0.930	2.401	2.080	86.6	4.0	9.602
	D8	2.972		3.871					
Un25	E8	3.189		1.973	1.505	0.662	44.0	4.0	6.020

Goto page 161

Witnessed & Understood by me, [redacted] Date Invented by [redacted]

Recorded by [redacted]

TITLE

From Pa

Book
page
S. Phi
Nous

MeanValue

Witnes

TITLE Human Endostatin Elisa

Project No. 414

Book No. 1060

From Page No. 160

[redacted]

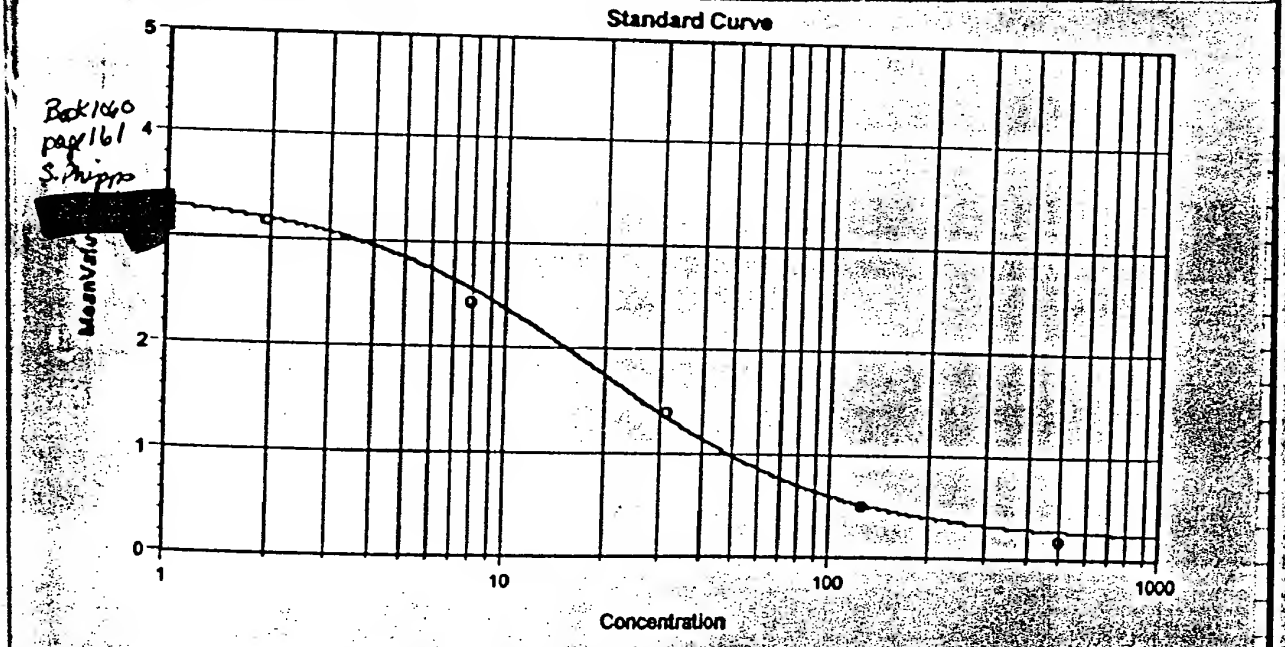
	F8	3.295	1.037						
Un26	G8	3.272	1.242	4.930	5.215	105.8	4.0	19.718	
	H8	2.496	8.617						
Un27	A9	0.421	173.886	149.016	35.172	23.6	4.0	596.063	
	B9	0.518	124.145						
Un28	C9	0.631	92.676	87.254	7.668	8.8	4.0	349.015	
	D9	0.688	81.832						
Un29	E9	3.120	2.576	2.236	0.480	21.5	4.0	8.946	
	F9	3.198	1.897						
Un30	G9	3.329	0.728	1.028	0.425	41.3	4.0	4.113	
	H9	3.263	1.328						
Un31	A10	3.357	0.454	0.305	0.210	69.0	4.0	1.221	
	B10	3.384	0.156						
Un32	C10	3.218	1.719	1.288	0.609	47.2	4.0	5.154	
	D10	3.315	0.858						
Un33	E10	3.301	0.982	1.133	0.213	18.8	4.0	4.533	
	F10	3.268	1.284						
Un34	G10	3.117	2.597	2.181	0.588	26.9	4.0	8.726	
	H10	3.213	1.766						

[redacted]

Book 1060 pgs 161
S. Phipps

R - Outside standard range

Mean Adjusted Result: 77.18



Book 1060 pg 161
S. Phipps

[redacted]

	y=(A-D)/(1+(x/C)^B)+D:	A	B	C	D	R^2
Std (Standards: Concentration vs MeanValue)		3.395	1.178	19.115	0.2	0.988

Witnessed & Understood by me,

Date [redacted]

Invented by

Recorded by

Date [redacted]

Project No. 414
Book No. 1060 TITLE AV4 human Endostatin CVL Amplification /

From Page No. [redacted] Particles/ml of AV8MhandLx

Collected supernatant from AV4 human endostatin 4 well - 4th amplification made with 3 plasmid System (293 cells). 3.75 ug DNA + 1.67 ug DNA 9 day pi.
Collected Sup from AV4 human endostatin - 3rd amplification made 3 plasmids in Clone 54 cells. 9 day pi.
Collected 2 wells each of mouse TPLi endostatin & human TPLi endostatin supernatant - from initial 3 plasmid transfection of AV4 on Clone 54 cells. (7 day pi.) - Stored @ 4°C for ELISA testing

Harvested 9 day amplified CVL from above samples AV4 human endo - 5 plasmid (293) 4th amplification on Clone 54 cells.
Harvested CVL from AV4 human endo - made 3 plasmid clone 54 9 day pi
Harvested initial CVL from transfection (7 day post transfection) of AV4 human + mouse TPLi endostatin made in Clone 54 cells using lipofectamine + 3 plasmids.

Freeze thawed above CVLS 5X - reamplified 2nd AV4 human endo - 5th amplification on 10 cm dish clone 54 cells added 6 ml added 3.75 ml Amplified 1.67 ug DNA CVL AV4 human endostatin 5th amplification on 10 cm dish clone 54 cells.

Amplified 1st time from original transfection CVL of mouse + human TPLi endo AV4 made in 3 plasmid clone 54 cells on 4 wells of clone 54 passage 14 cells. Added 0.5 ml / well.

Split Chang cells p4 T150 1:10
Split 293 cells p24 T150 1:20

Took 2 (50 ul) aliquots of AV8MhandLx virus & 1 (100 ul) & 1 (200 ul) aliquot to look @ particles/ml & make Viral genomic DNA.

100 ul Virus — 10 min @ 56°C with shaking
900 ul Viral concentrator buffer — 10 min @ room temp.
Read 260 nm OD

To Page No.

Witnessed & Understood by me. [redacted] Date [redacted] Invented by [redacted] Recorded by [redacted] Date [redacted]

From Page No. [redacted]

To make viral genomic DNA for LX
175ul virus
175ul 10xTES
7.5ul proteinase K incubate@ 56°C 3½4hr with shaking
Phenol/Chloroform extract 1x; isoamyl/Chloroform extract 1x
Ethanol ppt. leave Over night@ -20°C (1ml EtoH + 200ul DNA.

ODreadings for particles are below. I don't understand why 2nd aliquot of 10ul gave such high ODreading. I will proceed particles on @ least 1 more aliquot & compare to these results.

BECKMAN DU-600 Date: [redacted] Time: 12:58

Book 1060
page 165
S. Phipps
[redacted]

Nucleic Acid
ReadSamples Method SaveClear Print Quit

Results file: A:\WORK_RES Method name: A:\DEFAULT

Assay type: General Ratio and Concentration Units: ug/ml
Formula setup: VIEW Background Correction: [No]
Sampling device: None Concentration: [Yes]
Read average time: 0.50 sec Peak Pick: [No] 1 1

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml	
1	0.0701	0.0670	1.0461	0.9559	0.0000	0.1751	(1)
2	0.0711	0.0679	1.0475	0.9546	0.0000	0.1778	
3	0.2508	0.2535	0.9894	1.0107	0.0000	0.6270	(2)
4	0.2562	0.2575	0.9951	1.0049	0.0000	0.6406	
5	0.0547	0.0533	1.0255	0.9751	0.0000	0.1367	(3)
6	0.0560	0.0523	1.0715	0.9332	0.0000	0.1401	
7							

OD reading of AVBM hand LX (AV3 human endostatin Virus
Lot # 5526-98

0.0706 x 10 x 1.12×10^{12} particles/ml = 7.9×10^{11}

0.05535 x 10 x 1.12×10^{12} particles/ml = 6.2×10^{11}

I did swipe tests room 110

Based on aliquot #1 + 3 the average particle/ml is

7.05×10^{11} particles/ml

To Page No.

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by [redacted]

Recorded by [redacted] Date [redacted]

TITLE AVBMkendLX Viral DNA/genomic DNA Isolation of   Project No. 414
Book No. 1060

From Page No. [redacted]

Mouse+Human TPLintron Endostatin

Finished Puregene genomic DNA isolation of mouse+human TPLi-endostatin and Endostatin transient transfections from page 163

Precipitated AVIBMkendLX viral genomic DNA from 165 → centrifuged 5min. Washed 1X 70% EtOH air dried pellet. Resuspended 50ul 1X TE.

Split S8 cells passage 25 to make 50 TCID50 plates (96 well plates)
Used 1 roller bottle
172 } 174.5x10⁴ cells/ml
177 }

5×10^4 cells/ml x 1000ml = 5×10^7 cells / 174.5x10⁴ cells/ml   Need 28.65ml cells

+ 4ml dexamethasone
Plated 200ul/well for 1×10^4 cells/well (96 well plate)

Plated 4 6 well dishes
3.5x10⁵ cells/well Need 8.75x10⁴ cells/ml x 110ml =
9.62x10⁶ cells / 174.5x10⁴ cells/ml
Need 5.5ml of cells + 105ml media + 0.33ml dexamethasone

Split 1 T150 1:20

Sandrina Phipps [redacted]

[redacted]

Ran PCR products 0.8% agarose gel 10ul of each run.
Lane 1 mwt λHindIII φXHaeIII
Lanes 2-19 → 1a thru SQ1a
(#1) #18
Gel picture page 166
Lane 20 - 1Kb ladder

The correct fragment ≥ 1Kb.
Bottom gel broke next to molecular wt marker is 0.4ug pAVE1aLX2 & to the left of that 1.2ug pAVE1aLX2 & no DNA control well

To Page No. 168

Witnessed & Understood by me, [signature] | Date [redacted] | Invented by | Date [redacted]
Recorded by [signature]

Project No. 414
Book No. 1060
TITLE PCR Amplification of Homologous Recombinants

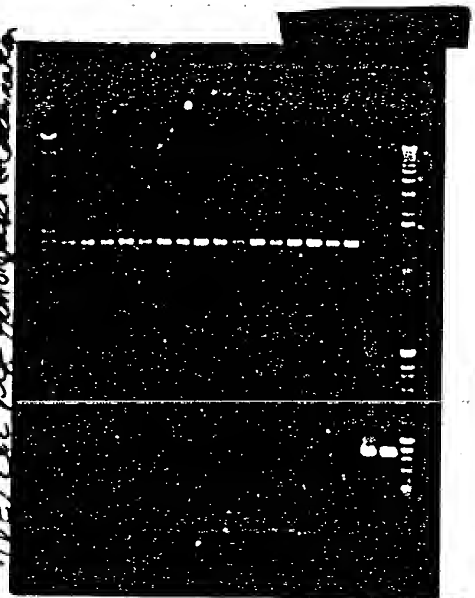
From Page No. 167

[redacted]

EXP 1-4. / Amplification of AV4 Human Endostatin

Lanes 18-19 top gel chart SQ1a duplicates Which should not amplify with these PCR primers YNC572/CH12

[redacted]



Book1060 page 168 S. Phipps [redacted]

Collected 5th amplification CVL of AV4 human endostatin upside (293 5plex) then transferred to Clone 54 for 4 amplifications.
Collected 10cm plate - took 4ml after Centrifuging to run on human endostatin elisa kit (from page 164)
Froze CVL @ -70°C The CVL was CPE @ 4 days post infection

To Page No.

Witnessed & Understood by me, [signature] | Date [redacted] | Invented by | Date [redacted]
Recorded by [signature]

TITLE E2
From Page N
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Samp
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Witnessed & Un

Project No. 414
Book No. 1060 TITLE TCID50 Titer of AV3BmhendLx Virus / Tissue Culture

From Page No. [redacted]

Split Hep 3B cells 2 T_{150} 1:2 & 1:20 pass 61 EMEM + 10% FBS
Split Chang cells T_{150} 1:40 pass 5 after freeze EMEM + 10% FBS
Split 293 T_{150} 1:40 pass 25
Split LS180 T_{150} 1:40 pass 4 after thaw

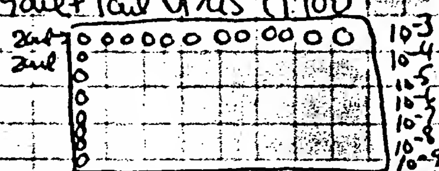
Split Clone 54 cells passage 15
104 }
86 } 88 x 10^4 cells/ml
74 }

Need 1 x 10^5 cells/ml x 26 ml = 2.6 x 10^6 cells / 88 x 10^4 cells/ml
Need ~~2 3ml~~ SIP 3ml of cells + 78ul dexamethasone

Need 4 x 10^5 cells / 10cm plate
4 x 10^5 cells/ml x 12 ml = 4.8 x 10^6 cells / 88 x 10^4 cells/ml
5.45ml + 36ul dexamethasone

Split 2 T_{150} 1:30 put 90ul dexamethasone in 30ml
Split 1 T_{150} 1:20 to carry passage 15
Split Hela cells 1:50
Split Saos2 cells passage 2 from freeze T_{150} 1:10
Diana Moffat will freeze these cells Saos2 down today approx 10 Vials

Made serial dilutions of AV3 BmhendLx Virus Lot SSP6-98
Diluted virus 1:100 then made 10 fold dilutions to put down
96 well plate 990ul + 10ul virus (1:100)
Add 20ul/well with repipetor



Did duplicate plates from 2 sets of serial dilutions

Also mad to 10ul aliquots of virus + 90ul concentration lysis buffer to viral particle/ml calculation. Incubate 10min @ 56°C then @ room temp for 10min. Read 260nm OD reading. OD Readings were too low need to repeat

To Page No.

Witnessed & Understood by me, [signature] Date [redacted] Invented by [signature] Recorded by [signature] Date [redacted]

TITLE Human Endostatin Assay of Lentivirus / Project No. 414 Book No. 1060

From Page No. [redacted]

/ AV4 + AV3 BMhendLX

Split Hep3B cells 1:50 for Theresa Chen 1 and of 2 T150 passage 62
Split Chang cells 1:50 T150 → passage 6.

Set up endostatin Elisa (human) Tested lentivirus supt for endostatin heparin purified AV3 BMhendLX virus + AV4 BMhendLX virus supernatants

Made 1:2 & 1:4 dilutions for all samples except the heparin column purified AV3BMhendLX made 1:10, 1:50, 1:250, 1:500 dilutions

Followed protocol page 1059/106
For 1:2 use 200ul sample + 200ul diluent #1
For 1:4 use 100ul sample + 200ul diluent 1 + 100ul diluent 2
For 1:10 use 40ul purified AV3BMhendLX in 260ul diluent #1 100ul diluent #2
1:50 use 8ul + 92ul diluent extra
1:250 use 16ul of 1:10 + 84ul extra diluent #1
1:500 use 8ul of 1:10 + 92ul extra diluent #1

Samples
~~1 AV3 TPLi endLX supt transfection 3plasmid 58 12day p.i.~~ [redacted]
2 AV4 TPLi endLX supt Clone54 3plasmid 7day p.i. [redacted] 1st supt from transfection
9. AV4 TPLi BMhendLX Clone54 3plasmid 7day p.i. [redacted] " " "
~~4 AV4 TPLi BMhendLX Clone 54 5th round amplification (293 plasmid) 3.75ug 4th day p.i.~~ [redacted]
3 AV4 human endo Clone 54 cells 3rd amp [illegible] 3 plasmid Clone 54 9 day p.i.
6 AV3 TPLi endLX supt transfection 3plasmid 58 12day p.i. [redacted]
7 AV4TPLi endLX 8day p.i. supt from initial transfection 5plasmid 293 [redacted]
8 AV3 TPLi BMhendLX sup 1 transfection 5plasmid 58 10day p.i. [redacted]
~~9 AV4TPLi BMhendLX supt after initial transfection 293 5plasmid 8day p.i.~~ [redacted]
10 Clone 54/passage amp 4 AV4 human endostatin (5plasmid 293) 9day p.i. [redacted]
11 (2mg/ml) AV3 human endo [redacted] - dialyzed
Chris Wysocki Set up lentivirus supernatants 8 samples
(2 dilutions 1:2, 1:4) 8 samples are duplicates of one another
① AV4 BMhendLX 1.67ug DNA (293 5plasmid 3rd amp [redacted] 9day p.i.
5 AV4TPLi BMhendLX supt after initial transfection 293 5plasmid [redacted] 8day p.i.

To Page No. 180

Witnessed & Understood by me, [signature] Date [redacted] Invented by [signature] Date [redacted]
Recorded by [signature]

Project No. [redacted]
Book No. [redacted] TITLE [redacted]

From Page No. 172 [redacted]

[redacted]

[redacted] Book 1000 pg 150 [redacted]

	1	2	3	4	5	6	7	8	9	10	11	12
A	[redacted]											
B												
C												
D												
E												
F												
G												
H												

Book 1000 pg 150 S. Plugs [redacted]

To Page No. 181

[redacted] Recorded by [redacted]

TITLE Human Endostatin Assay

Project No. 414

Book No. 1060

From Page No. 180 [redacted]

Book1060 page 181 Plate#1 S. Phipps [redacted]

1 2 3 4 5 6 7 8 9 10 11 12

A B C D E F G H [redacted]

Formula: L1

UnknownsDilution Book1060 page 181 Sandra Phipps [redacted]

Sample	Wells	Values	R	Result	MeanResult	Std Dev.	CV%	Dilution	Adj.Result
Un01	E2	2.874	R	Range?	0.231	0.000	0.0	4.0	0.925
	F2	2.768		0.231					
Un02	G2	2.501		2.503	1.434	1.512	105.4	2.0	2.869
	H2	2.752		0.365					
Un03	A3	3.090	R	Range?	Range?	Range?	Range?	4.0	Range?
	B3	2.830	R	Range?					
Un04	C3	2.877	R	Range?	Range?	Range?	Range?	2.0	Range?
	D3	2.984	R	Range?					
Un05	E3	2.502		2.490	2.490	0.000	0.0	4.0	9.960
	F3	2.866	R	Range?					
Un06	G3	2.623		1.439	1.439	0.000	0.0	2.0	2.878
	H3	2.795	R	Range?					
Un07	A4	2.917	R	Range?	Range?	Range?	Range?	4.0	Range?
	B4	2.887	R	Range?					
Un08	C4	0.313		263.297	274.047	15.203	5.5	2.0	548.094
	D4	0.303		284.797					
Un09	E4	0.536		97.567	97.508	0.083	0.1	4.0	390.033
	F4	0.537		97.450					
Un10	G4	2.583		1.780	2.337	0.788	33.7	2.0	4.674
	H4	2.458		2.894					
Un18	A5	2.954	R	Range?	Range?	Range?	Range?	4.0	Range?
	B5	2.863	R	Range?					
Un13	C5	2.853	R	Range?	Range?	Range?	Range?	2.0	Range?
	D5	2.825	R	Range?					
Un13	E5	2.830	R	Range?	Range?	Range?	Range?	4.0	Range?
	F5	3.010	R	Range?					
Un14	G5	2.808	R	Range?	Range?	Range?	Range?	2.0	Range?
	H5	2.805	R	Range?					
Un15	A6	2.958	R	Range?	Range?	Range?	Range?	4.0	Range?
	B6	3.013	R	Range?					

media

1 AV Bm ... 1.6 μg
1 9 day 3' amp.

2 AV ... clone 54
1st ... transfection
2

3 AV4 ... 3' amp
3 Clone 54 / clone 54

4 (469 ng/ml)
AV4 ... Shamp
4 293 3.75 μg (10 cm dish)

5 AV4 ... 5' plasmid
5 293

6 AV3 ... 3' plasmid
6 S8

7 AV4 ... 5' plasmid
7 293

182

Witnessed & Understood by me, [signature]

Date [redacted]

Invented by

Recorded by Sandra Phipps

Date [redacted]

182

Pr ject No. 414
Book No. 1060 TITLE Human Endostatin Assay

From Page N . 181 [redacted]

Un16	C6	2.685		0.916	0.715	0.285	39.9	2.0	1.430	8
	D6	2.734		0.513						
Un17	E6	2.664		1.090	0.725	0.516	71.2	4.0	2.900	8
	F6	2.752		0.360						
Un18	G6	0.550		93.705	91.411	3.244	3.5	2.0	182.822	8
	H6	0.568		89.117						
Un19	A7	0.990		36.823	37.318	0.701	1.9	4.0	149.273	9
	B7	0.974		37.814						9
Un20	C7	0.808		51.133	51.723	0.835	1.6	2.0	103.446	
	D7	0.796		52.314						10
Un21	E7	1.320		22.139	18.309	5.417	29.6	4.0	73.234	10
	F7	1.616		14.478						
Un22	A8	0.219		806.772	688.457	167.322	24.3	10.0	6884.573	
	B8	0.238		570.143						11
Un23	C8	0.753		57.136	53.852	4.645	8.6	50.0	? 2692.576	11
	D8	0.813		50.567						
Un24	E8	1.256		24.252	23.207	1.478	6.4	250.0	5801.704	11
	F8	1.320		22.161						
Un25	G8	1.609		14.613	15.496	1.249	8.1	500.0	7748.092	11
	H8	1.530		16.380						
Un26	A9	2.699		1:2 0.802	0.802	0.000	0.0	2.0	1.604	
	B9	3.066	R	1:4 Range?						
Un27	C9	2.811	R	1:2 Range?	Range?	Range?	Range?	4.0	Range?	
	D9	2.958	R	1:4 Range?						
Un28	E9	1.428		1:2 18.945	12.214	9.519	77.9	2.0	24.428	
	F9	2.204		1:4 5.483						
Un29	G9	1.561		1:2 15.671	10.542	7.254	68.8	4.0	42.167	
	H9	2.211		1:4 5.412						
Un30	A10	2.885	R	1:2 Range?	Range?	Range?	Range?	2.0	Range?	
	B10	2.962	R	1:4 Range?						
Un31	C10	3.142	R	1:2 Range?	Range?	Range?	Range?	2.0	Range?	
	D10	2.986	R	1:4 Range?						
	E10	2.975	R	1:2 Range?						
	F10	3.007	R	1:4 Range?						
Un32	G10	3.131	R	1:2 Range?	Range?	Range?	Range?	4.0	Range?	
	H10	[illegible]	[illegible]	1:4 [illegible]						

[redacted] 4:59:40 PM HUENDO112398SSP.pda

2.165 ng/ml
AV3 TRi BmHand LX 3 plasmid 58
166 ng/ml
AV4 TRi BmHand LX 54 3 plasmid
88.34
AV4 passage 4 5 plasmid 293 Clone 54
AV3 BmHand LX purified - dilution made wrong
Avg. 6811
6.8 ug/ml

Chris Wysock's samples
Lentivirus supernatants [redacted]

Book 1060 page 182
S. Phipps

Book 1060 pg 182

Standards (ng/ml) [redacted] S Phipps

Sample	Concentration	BackCalcConc	Wells	Values	MeanValue	Std.Dev.	CV%
St01	500.000	Range?	A1	0.145	0.176	0.043	24.5
		1123.306	B1	0.206			
St02	125.000	133.936	C1	0.444	0.496	0.074	15.0
		94.118	D1	0.549			
St03	31.250	24.320	E1	1.256	1.131	0.177	15.6
		35.833	F1	1.007			
St04	7.810	8.419	G1	1.970	1.940	0.043	2.2
		9.300	H1	1.909			
St05	1.950	0.927	A2	2.684	2.559	0.177	6.9
		3.117	B2	2.434			
St06	0.000	Range?	C2	2.956	2.793	0.230	8.2
		1.370	D2	2.631			

To Page No. 183

[signature] [redacted]

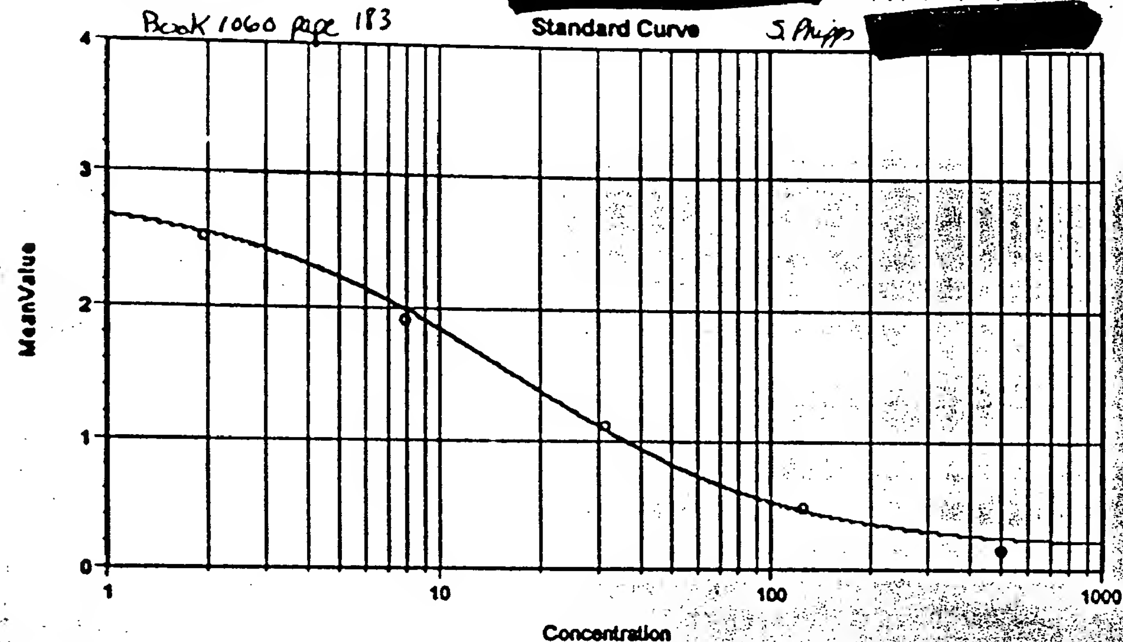
Recorded by Sandra Phipps [redacted]

Da[redacted]

TITLE Human Endostatin Assay — **Project No.** 414 — **Book No.** 1060

From Page N 182

[redacted]



The heparin purified AV3BmhendLx virus (6.8 ug/ml)

The AV4 BmhendLX virus amplifications
AV4 BmhendLX amplification passage (4) 88.34 ng/ml
AV4 BmhendLX amplification passage (5) 469 ng/ml

The AV4 BmhendLX TPi clone 54 (3 plasmid) 7 day p.i. from original transfection Supt
now has 166 ng/ml of human endostatin
None of other AV3 & AV4 TPi endostatin viruses have produced much endostatin
Sandra Phipps [redacted]

[redacted]

Split S8 cells passage 28 1:50
" SaOS2 cells passage 3 1:50
" LS180 cells passage 3 1:50
" Hela cells 1:50
Threw away 293 cells because of high passage
Jane Xu split Clone 54 cells for me.

To Page No. 184

Witnessed & Understood by me, [signature] — Date [redacted] — Invented by [signature] — Date [redacted]
Recorded by Sandra Phipps

Project No. 414
Book No. 1060 TITLE Collecting Amplified CVL of AV4 & AV3 TPLi

From Page No. [redacted]

BMH and LX

Titer of AV3 BmhandLx Virus

Split 293 cells 1 complete 1:10, 1:20 (2 T150) passage 10 from Dawn Kanda
Split 58 cells passage 12: June Line Split 1 complete into 6 well dishes @ 4x10^5 cells/well.

Collected Supt and CVL from AV4TPLi and Lx & AV4TPLiBmhandLx
1st Amplification clone 54 cells (293 Spleen d system) - 10 day p.i.
Collected Supt and CVL from AV3TPLi and Lx & AV3TPLiBMhand Lx
1st amplification 58 cells (3 plasmid 58 cells) 11 day p.i.
Froze CVL @ -70C after Snap freezing.
Stored Supt @ 4°C.

Read TCID50 titers of AV3 BMhandLx Virus

Book 1060 page 186
S. Phipps

Read [redacted] 1:100

Sample: AV3 BmhandLx Virus (B)

	1	2	3	4	5	6	7	8	9	10	11	12
B												
C												
D												
E	+	+	+	+	+	+	+	+	+	+	+	+
F	+	−	−	−	+	+	+	+	−	+	+	+
G	+	−	−	−	−	−	−	+	−	−	−	−
H												

BX

[redacted] 1:100

Sample: AV3 BmhandLx Virus A

	1	2	3	4	5	6	7	8	9	10	11	12
B												
C												
D												
E	+	+	+	+	+	+	+	+	+	+	+	+
F	+	+	−	+	+	−	+	+	+	+	+	+
G	−	−	−	+	−							
H												

7.97x10^8
9.65x10^8
8.81x10^8

Titer for B - 7.97x10^8
A - 9.65x10^8
} Avg. titer 8.8x10^8 PFU/ml

To Page No.

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by

Record d by [redacted] Date [redacted]

From Page No.

[redacted]

1) use Hi Trap Heparin (pharmacia Biotech #17-0406-01) Column to process Supernatant (from Mammalian).

wash w/ 10 ml of PBS (using 10ml syringe)

↓

Loaded 100mL of Supernatant.

↓

wash w/ 10ml of wash buffer (50mM Tris-Cl PH 7.5, 100mM NaCl)

↓

Elutel the Heparin bound protein with 10ml Elute Buffer (50mM Tris-Cl PH 7.5, 1M NaCl, 20% glycerol)

↓

Collected fractions at 1ml/tube for 10 tubes

↓

protein Assay to determine protein Conc.

Assay type: General Ratio and Concentration
Formula setup: TIM1 unlabled Seal
Sampling device: Base
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No] 8

Sample ID	abs 280.0 nm	abs 280.0 nm	280.0 nm / 280.0 nm	280.0 nm / 280.0 nm	Protein ug/ml	Nucleic Acid ug/ml	
1	2.4230	2.4344	0.9953	1.0047	0.0000	0.0575	→ pooled tube #1 & #2, keep in 4°C.
2	2.7017	2.6965	1.0022	0.9240	0.0000	0.7544	
3	0.5550	0.4922	1.1276	0.8866	0.0000	1.3075	
4	0.2230	0.2772	1.1902	0.8402	0.0000	0.5240	
5	0.1785	0.1570	1.1372	0.8793	0.0000	0.4463	
6	0.1206	0.1206	1.0491	0.9532	0.0000	0.3164	
7	0.1421	0.1297	1.0952	0.9131	0.0000	0.3668	
8	0.0698	0.0778	0.8824	1.1333	0.0000	0.1719	
9	0.0516	0.0670	0.7706	1.2978	0.0000	0.1281	
10	0.0327	0.0547	0.6455	1.5491	0.0000	0.0818	

To Page No. 10

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by [redacted] Date [redacted]

Recorded by [redacted]

Project No. 414
Book No. 1127 TITLE: AV3hEndo

From Page No. 9

[redacted] Dialysis (desalt) of AV3hEndo

using dialysis cassette from pierce Slide-A-Lyzer (3.5kD) #98061975

dialysis 200ul of pool#1 & #2 (see p9) in 500ml of PBS, cold Room X 2hrs

[redacted] protein Assay of AV3hEndo

		①	②	③	④	⑤	⑥	⑦	8	9	10	11
0.8ml H_2O	ul	800	800	800	800	800	800	800	800	800	800	800
2ml Bio-Rad protein Assay dye #500-0006	ul	200	200	200	200	200	200	200	200	200	200	200
BSA (1ug/ul)	ul	0	2	4	6	8	10	15	2	4	2	4
								/Endo				

↓

Transfer 200ul to 96-well plate, each sample for 3 wells

↓

Read ABS at 595 nm

Experiment#1
#1 & #2 pooled PAV3hEndo desalted PAV3hEndo

Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	[redacted]										
B	-0.002											
C	0.001											
D	0	2λ	4λ	6λ	8λ	10λ	15λ	2λ	4λ	2λ	4λ	
E												
F												
G												
H												

Bsa 1ug/λ

• Endpoint
L1 595
AA Off
Calibrate On
Plate Last Read 12:08 PM [redacted]

Witnessed & Understood by me, [redacted] Date [redacted]

Invented by

Recorded by [redacted] Date [redacted]

From Page No. 10

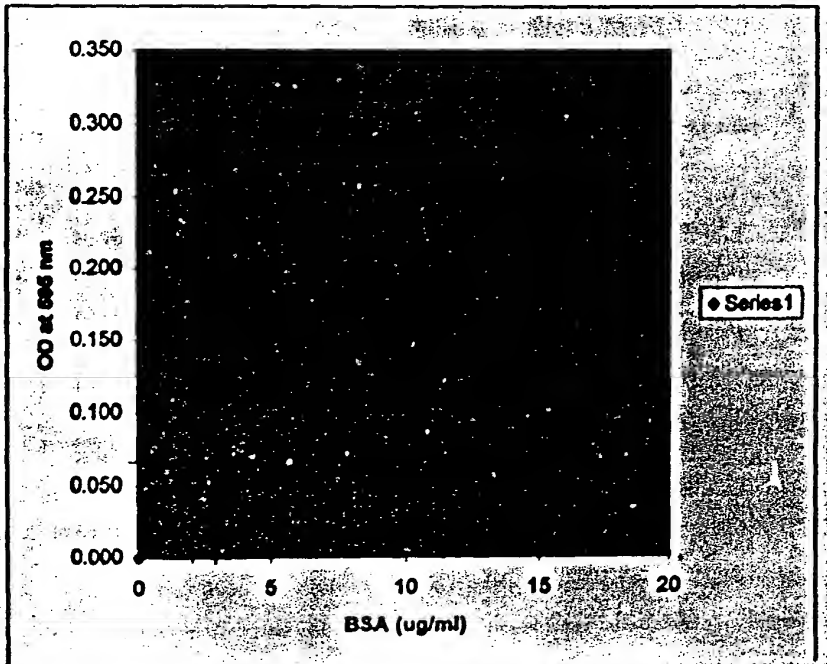
BSA(1mg/ml)	well 1	2	3	Average	
0	0.001	-0.002	0.001	0.000	
2µg	0.058	0.065	0.058	0.060	
4µg	0.106	0.105	0.103	0.105	
6µg	0.119	0.119	0.112	0.117	
8µg	0.19	0.191	0.186	0.189	
10µg	0.265	0.26	0.268	0.264	
15µg	0.334		0.294	0.314	
hEndo					
2nd u1	0.133	0.13	0.133	0.132	0.066
3rd u1	0.253	0.247	0.242	0.247	0.062
3rd u2 >desalt	0.101	0.102	0.098	0.100	0.050
4th u2	0.165	0.165	0.157	0.162	0.041

Keep in -70°C

3mg/ml — hEndo pooled #1 & 2

2mg/ml (diluted) — desalted hEndo

Keep in 4°C



To Page No. 12

Witnessed & Understood by me, [redacted] Date

Invented by

Recorded by [redacted] Date

Project No. 414
Book No. 1127   TITLE AV3hEndo

From Page No. 11   SDS-page : 4~20% pre-cast gel (Bio-Rad)

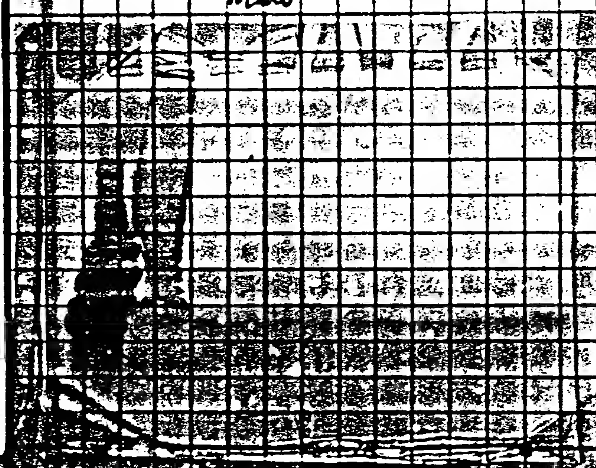
[redacted] AV3hEndo : desalted ~2mg/mL

Samples boiling : 105 ul dye + 105 ul PAV3endo
90°C x 3min

Gel I (For commassie blue Staining)
120V for ~1 hr

Gel II Load 20ul each Lane
For transfer PVDF 0.2u filter (Schleicher & Schuell)
Transfer at 100V for 1hr

hEndo



marker: ezprotein mid range blue prestained protein marker (life 7402)

SDS-gel
↓
Take the SDS-page gel, wash with ddH2O x 3 times
↓
Commassie stain (Pierce #98072460) for 1hr
↓
wash with ddH2O

Transfer procedure :
Soak PVDF membrane in methanol for a while
↓
Then soak in Western Transfer Buffer (keep in 4°C)
↓
TransferBlot (Bio-Rad)
See manual p7
gray panel + sponge + filter paper + gel + PVDF membrane (no bubbles in each layer)
↓
After transfer, the membrane will be stained with 0.1% commassie blue R250 in 40% methanol and 1% ~~[illegible]~~ acetic acid for 2min; destain in 50% methanol
wash c̄ ddH2O, 15 min per wash x 4 times

T Page No. 13

Witnessed & Understood by me,   Date [redacted]   Invented by   Date [redacted]
Recorded by

TITLE AV3hendo

Project No. 414

Book No. 1127

From Page No. 12

hendo PVDF Transfer

← 20kD

↓

Send protein (AV3hendo) to Midwest Analytical, Inc for protein Sequence

To Pag No.

Witnessed & Understood by me.

Date [redacted]

Invented by

Date

Recorded by

[redacted]

TITLE N-terminus protein peptide sequence Analysis of secreted human osteoblastic Project No. 414

Book No. 1080

From Page No.

[redacted]

[objectives]: To determine the cleavage product of the product from A13hEndo transduced Sf cells.

[Experiments]: Qin Li processed the conditioned medium of A13hEndo transduced Sf cells with Hepain Sepharose. Loaded the Heparin bound fraction pool on SDS-PAGE. The proteins were transferred to PVDF membrane and stained with Coomassie blue. The PVDF blot was sent to Midwest Analytical, Inc for N-terminus peptide sequence analysis.

[Results]:

MIDWEST ANALYTICAL, INC.
[redacted]
St Louis, MO 63123

[redacted] Notebook 1080 Page 129

[redacted]

Dr. Thomas Chen
Genetic Therapy, Inc.
99 Firefield Road
Gaithersburg, MD 20878

Dear Thomas:

Here are the chromatograms from the 20K band. The PTH standard was 20 pmol.

The primary sequence was APQQEALAMS.

The two secondary sequences matched your sequence, but started at different positions (LAMS... and MSMR...).

Please call me at 1-800-481-6790 if you have any questions about this run. Thanks again.

Sincerely,

David W. McCourt
[redacted]

Cheng-T. Chen

MIDWEST ANALYTICAL [redacted] Notebook 1080 Page 129
David McCourt 800-481-6790
St. Louis, MO 63123

Dr. Thomas Chen, Genetic Therapy

Sample: 20K [redacted]
Two bands were loaded for 10 residue N-terminal analysis.

File A3123[redacted]

1	A	ST	N	L	A
2	P	A	S	K	
3	Q	N	N	P	
4	Q	S	E	K	
5	E	N	D		
6	A	R	F		
7	L	D	Q		
8	A	P	P / A		
9	N	Q	V		
10	S	P	L	A?	

E5 = 1.3 pmol D5 = 0.7 pmol

Thomas,

There was an unambiguous primary sequence and two secondary sequences.

The secondary components matched your sequence, but they started at different positions. The end of the sequence looks like QPV instead of QAG. I did not observe any increase in G at residue #9.

Cheng-T. Chen

To Page No. 130

Witnessed & Understood by me,
M. O'Reilly

Date [redacted]

Invented by

Date

Recorded by Cheng-T. Chen

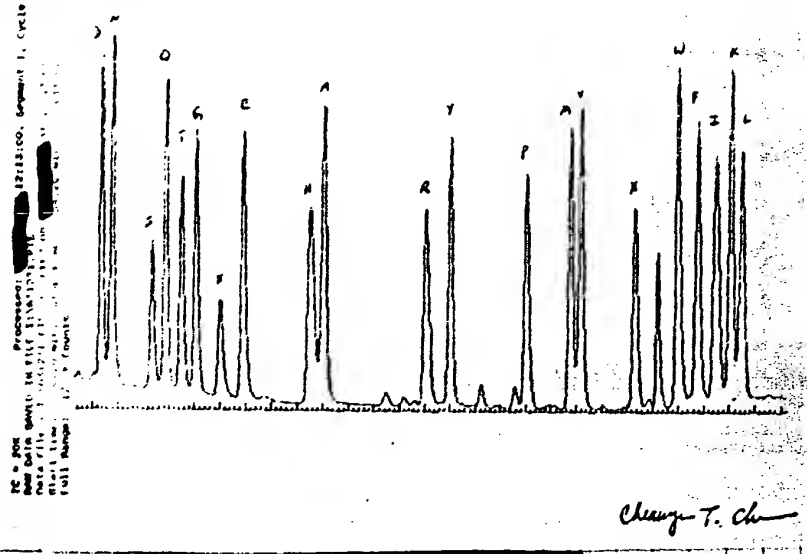
Date [redacted]

Project No. [redacted] 414

130 Book No. 1080 TITLE N-terminus peptide sequence analysis of secreted human [redacted]

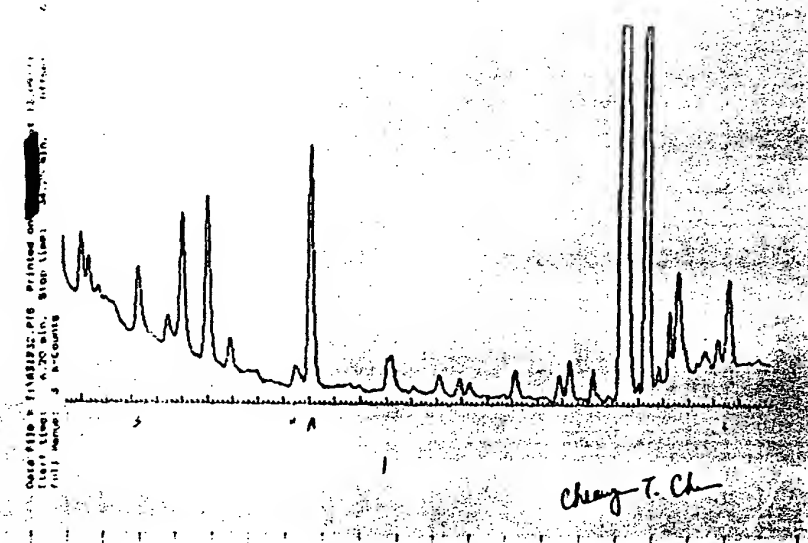
From Page No. [redacted]

Notebook 1080 page 130



Cheng T. Chen

Notebook 1080 page 130



Cheng T. Chen

To Page No. 131

Witness d & Und rstood by me, M. O'Reilly Date [redacted]

Inv nted by Date

Recorded by Cheng T. Chen [redacted]

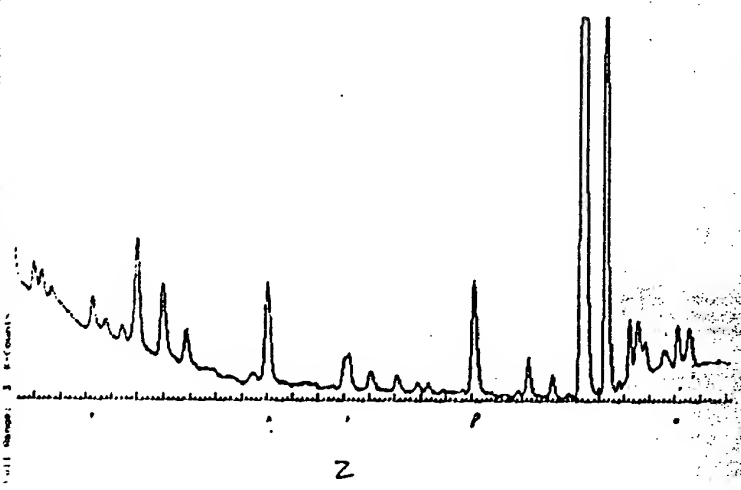
TITLE N-terminus peptide sequence analysis of secretin human intestinal Project No. 414

Book No. 1080

From Page No. 130

[redacted]

Notebook 1080 page 131

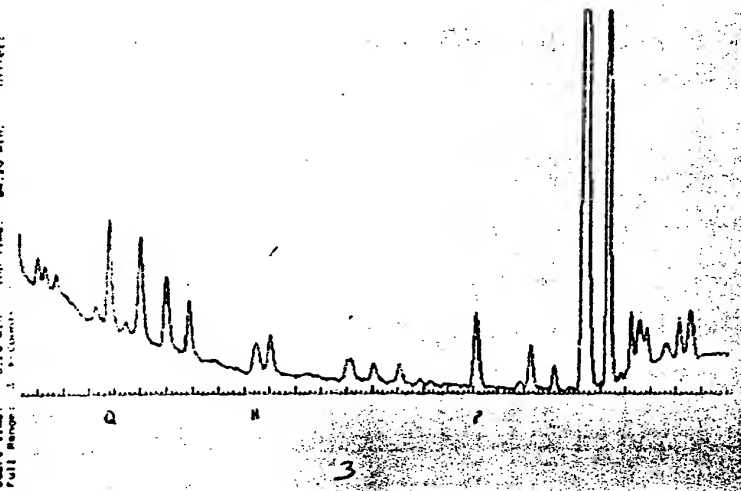


2

Cheung-T. Chen

[redacted]

Notebook 1080 page 131



3

Cheung-T Chen

To Page No. 132

Witnessed & Understood by m. O'Reilly

Date [redacted]

Invented by

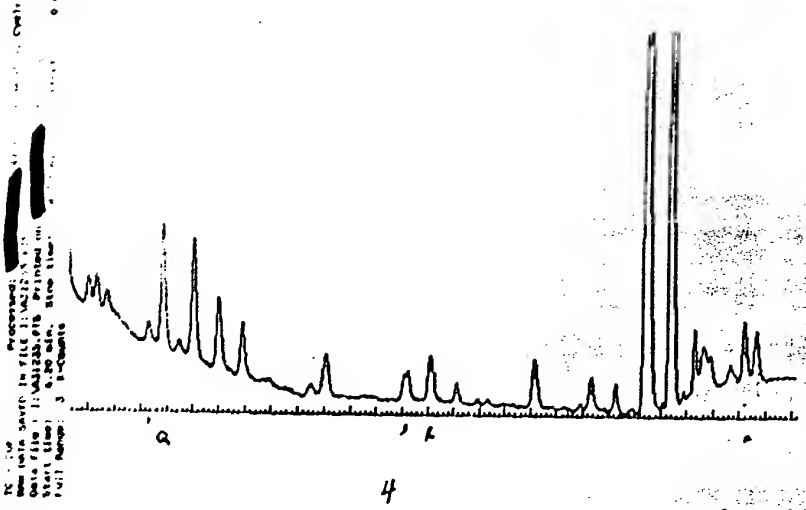
Date [redacted]

Recorded by Cheung-T. Chen

Project No. 414
Book No. 1880
TITLE N-terminus peptide sequence analysis of secreted human endostatin

From Page No. 13

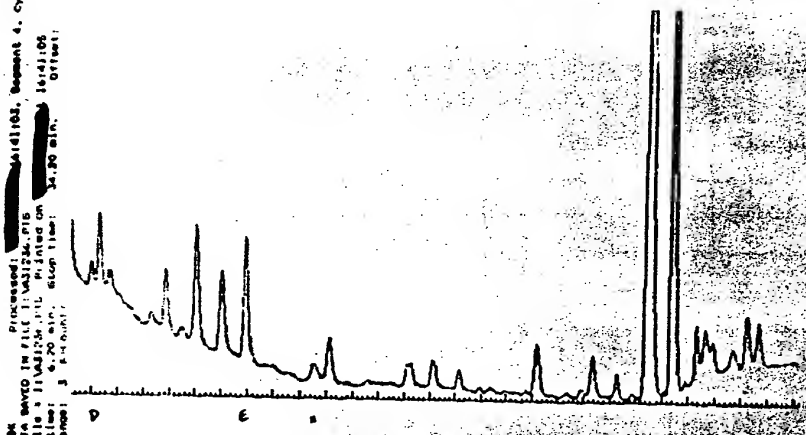
[redacted] [redacted] Notebook 1880 Page 132



4

Chuanyun T. Chen

Jan 4, 99 Notebook 1880 Page 132



5

Chuanyun T. Chen

To Page No. 13

Witnessed & Understood by me, M. O'Leilly

Date [redacted]

Invented by

Date

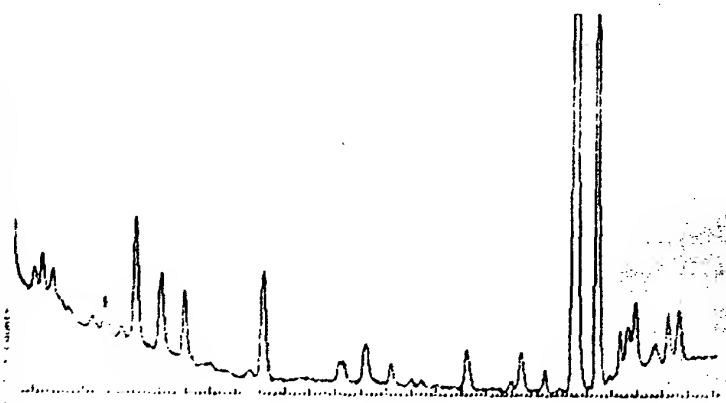
Recorded by Chuanyun T. Chen [redacted]

TITLE N-terminus peptide sequence analysis of human Endostatin Project No. 414
Book N . 1080

From Page No. B2

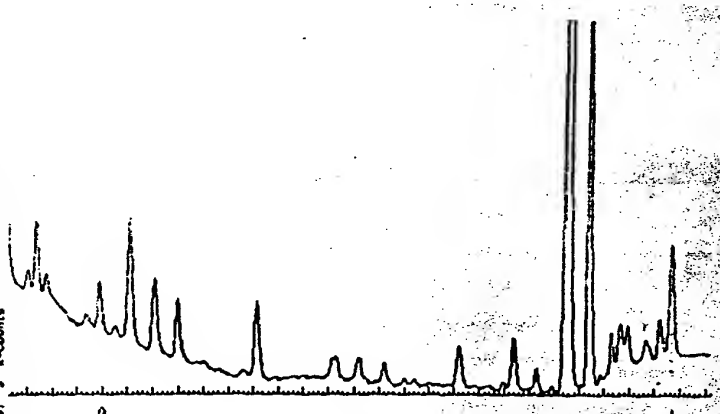
[redacted]

Notebook 1080 Page 133



Cheunyan T. Che

Jan. 4, 99 Notebook 1080 Page 133



Cheunyan T. Che

To Page No. B4

Witnessed & Understood by me,

M. O'Reilly

Date [redacted]

Invented by

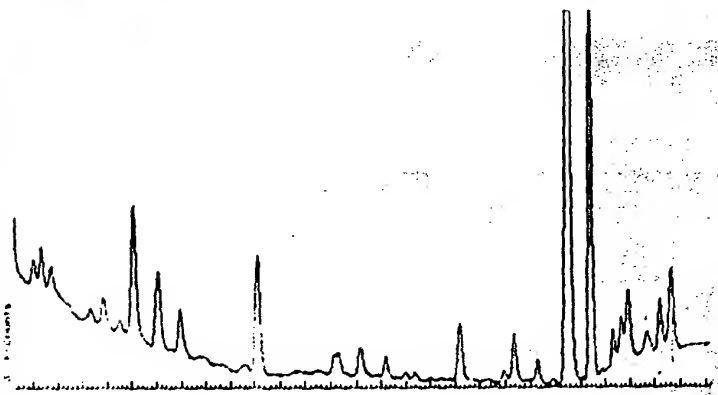
Recorded by Cheunyan T. Che

Date [redacted]

Project No. 414
Book No. 1080 TITLE N-terminus peptide sequence analysis of secreted human Galectin

From Page No. 133

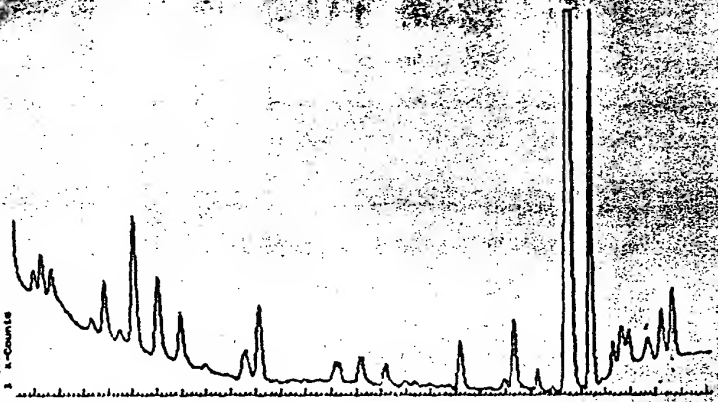
[redacted] Notebook 1080 page 134



8

Cheongju T. Cho

[redacted] Notebook 1080 page 134



9

Cheongju T. Cho

To Page No. 135

Witn s d & Understood by me.
M. O'Reilly

Date [redacted]

Invented by

Date

Record d by Cheongju T. Cho

Date [redacted]

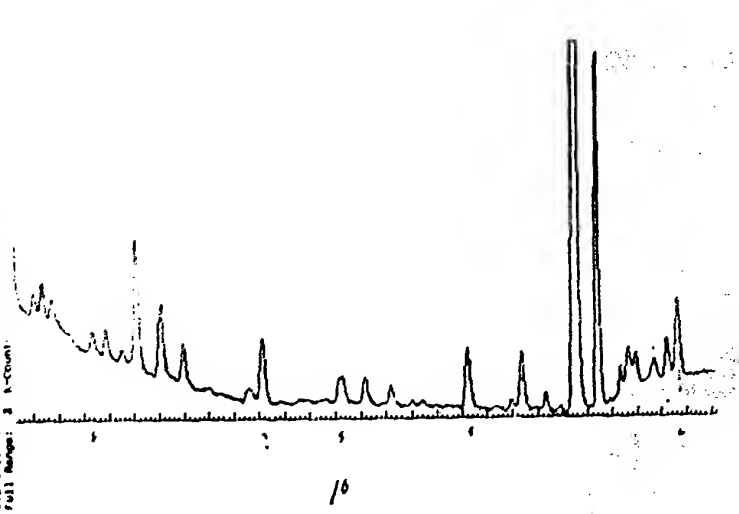
TITLE N-terminus peptide sequence analysis of secreted human Endostatin

Project No. 414

Book No. 1080

From Page No. 134

[redacted] Notebook 1080 page 135



Cheunjee T. Che

[redacted] Notebook 1080 page 135

50%

25%

25%

MRAWIFFLLCLAGRALAAPQQEALAHSHRDFQPVL----------

Human BM40 Signal **Human Endostatin**

Cheunjee T. Che

[Summary]
The sequence result showed expected peptide sequence which matched well with the published sequence. However, the cleavage site occur at 3 different locations whether or not the additional residues from signal peptide affect function will have to be tested

T Page No.

Witnessed & Understood by me,
M. O'Reilly

Date [redacted]

Invented by

Date

Recorded by Cheunjee T. Che

Date [redacted]